SOME CHEMICAL AND PHYSIOLOGICAL STUDIES ON THE NATURE AND TRANSMISSION OF "INFECTIOUS CHLOROSIS" IN VARIEGATED PLANTS[1]

EVERETT FOGG DAVIS

Assistant Plant Physiologist, Virginia Agricultural Experiment Station
Formerly Instructor in the Henry Shaw School of Botany of Washington University

INTRODUCTION

Experiments relating to the phenomena of the contagious nature of certain variegated varieties, when held in graft with a pure green variety of the species, have been known for over two hundred years. The earliest information upon the subject can not be considered reliable for the reason that the observations were recorded by the practical nurserymen, who too frequently failed to consider other possibilities for the appearance of new variegations than their transmission through grafts.

In regard to the contagious nature of the disease, a discussion of the literature must be concerned chiefly with the critical investigations performed by Lindemuth and Baur between the years 1904 and 1908. Very recently, however, Rischkow has attempted to reinvestigate the transmissibility of variegations in certain varieties of *Evonymus japonica*. The recorded experiments and observations of the former two investigators will form the basis for the present discussion of the literature. After considering the results of early investigators in the light of the general advances which have been made in recent years in the study of the mosaic diseases of plants, particularly in the matter of methods and of standardization of technic, the author has repeated certain aspects of these early attempts. From investigations with varieties of *Abutilon* and *Evonymus* new data were obtained which are included in the present paper.

In addition to my efforts to repeat the experiments of Baur, i. e., unsuccessful attempts to induce infection by inoculating

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healthy plants with the juices expressed from the leaves of chlorotic individuals and successful attempts to transmit the infection by means of grafts, certain experiments were performed with a view to determine facts concerned with the following:

I. Genesis of the chlorotic areas under the various conditions of light intensity, length of day, and concentration of carbon dioxide, as well as under conditions where the effects of light of different qualities may be observed.
II. Nature of the cellular environment of the green and variegated tissues of leaves held under the conditions mentioned above, determined by employing the following methods:
    a. Histological examinations of living and fixed leaf material.
    b. Determination of hydrogen-ion concentrations by the quin-hydrone electrode and the indicator methods.
    c. Analysis for total acidity by electrometric titrations.
III. Quantitative changes in the pigments and in the chlorophyll a/chlorophyll b and carotin/xanthophyll ratios in varieties infected with contagious variegations.

DISCUSSION OF THE LITERATURE

Aside from anatomical distinctions, infectious chloroses may be distinguished from non-infectious chloroses by the fact that the causal agency, which for the sake of convenience is commonly referred to as a virus, can be transmitted from a chlorotic (variegated) plant to a green plant. In the case of the non-infectious type the variegations can be perpetuated either by means of inheritance through the seed or by the vegetative reproduction of variegated plant parts. In some infectious chloroses transmission can be readily obtained by the inoculation of infectious plant juices into healthy plants. It has been shown that some species of insects are effective carriers of the virus in the case of certain chloroses. Chloroses which can be transmitted by the inoculation of the infected juice fall into the group of mosaic diseases. Still other chloroses can be transmitted by graft union of chlorotic and green parts, but they have not as yet

been successfully transmitted by inoculation. These have been referred to as "infectious chloroses," and it is to this latter group that the present paper is devoted.

Early Literature.—Historically, the transmission of a leaf variegation to the leaves of a stock or scion has been known since the successful graft experiments performed and reported by Wats in Kensington, 1700, John Lawrence in England, 1715, and Bradley, 1726. Practically, however, this line of research gained impetus in 1868, when a single plant of *Abutilon striatum* Dicks., imported from the East Indies to England, was found to possess a very attractive foliage spotted with green and yellow. Because of its beautiful leaves, an economic demand arose which caused it to be widely reproduced vegetatively in both England and France.

In 1869, Lemoine, in Nancy, made the first observations on the transfer of this leaf variegation to other species of *Abutilon* by grafting. In the course of the same year Morren ('69) wrote:

> "Les expériences [graft transfers] dont nous venons de relater les resultats ont été repetées plusieurs centaines de fois pendant le cours de l'année 1869 par M. Q. Wiot, directeur de l'établissement d'horticulture de Jacob Makoy et Cie, à Liège . . . Elles établissent, en effet, cette fois d'une maniere incontestable, la transmissibilité de la panachure du feuillage d'une plante à une autre par une sorte d'inoculation."

Beyond recognizing it as a disease of the so-called "green chromules" transmissible from plant to plant by means of grafting, Masters ('69), Morren ('69), Sageret ('69), Bouché ('71), Lindemuth ('78), and others knew little concerning the true nature or cause of the disorder. As early as 1899, Beijerinck thought it to be like the mosaic of tobacco, and he believed that it belonged in the same class of infectious diseases.

Masters ('69) established the fact that certain species other than the Malvaceae were also capable of transmitting or of receiving similar communicable diseases. He found a white variegated variety of *Jasminum officinale* which transferred its variegated appearance to the green stock, *Jasminum revolutum*. This established a new variety, *Jasminum revolutum foliis aureo variegatis*. Concerning it, Morren reported that if grafted back upon the white variegated variety of *Jasminum officinale*, it would regularly infect it and could thereby produce the variegated characters of "*aureo-variegatis*" upon it.

His work was followed by Bouché ('71), who grafted scions of a yellow and of a white variety of *Evonymus japonica* on the side of the stems of two green specimens. He observed later that these green plants produced twigs which bore clear traces of a whitish venation. He concluded that this change is to be considered as an infection resulting from an interchange of the sap from the white variegated scion. Lindemuth, however, thought it more probable that infection resulted from the scion of the yellow variety instead of the white. Morren ('69) found that infection could be brought about in some cases by introducing a petiole, with a variegated leaf blade attached, into an incision in the bark of the succulent stem of a plant bearing green leaves.

Chief among the early investigators interested in the solution of this problem was Lindemuth, who showed that in the genus *Abutilon* there are species and individuals having varying degrees of susceptibility to the infection. Light was found to favor the development of the mottled chlorosis in the Malvaceae, and high fertility was proved to be an additional factor favoring bright-colored variegations.

It was not until 1904, when Baur began his researches upon the nature of the *Abutilon* infection, that there was advanced a general concept relating the infectious chloroses to the virus diseases such as those of tobacco, potato, sugar cane, aster ("yellows"), and probably likewise the "yellows" of peach.

GENERAL PHYSIOLOGY AND PATHOLOGY OF VARIEGATIONS

PHYSIOLOGY OF NON-CONTAGIOUS VARIEGATIONS

The fundamental distinctions between non-infectious variegations of the mutant type and infectious variegations of the infectious disease type were recognized by but few of the early authors. Among those who did make this distinction were Vöchting and Lindemuth, although others classed all kinds of variegations in one group.

There have been mentioned in the literature of variegations several ways whereby variegated plants, leaves, shoots, and portions of each may be distinguished in physiological behavior from the clear green counterparts. The studies have had to do

with differences in the content of oxidizing enzymes, water, and turgidity, as well as differences in the concentration of chemical constituents. Where significant chemical differences have been found they compel the botanist to consider the variegated and the green foliage as individuals having very different physiological constitutions.

It has commonly been observed that variegated foliage and diseased plants break the winter rest period before healthy individuals. Lakon ('16, '17), working on the nature of the differences in the yearly periodicity of albino-leaved shoots of *Acer Negundo* L. and *Sambucus nigra* L., assumed with Klebs that this behavior may be accounted for by the inability of such leaves to accumulate and store photosynthetic products during the late summer. These authors believed that persistency in a state of rest, after external factors for growth are favorable, results from an inhibition of the enzymes by an accumulation of the end products for which they are directly responsible. Since in chlorophyll-deficient albino leaves there is no such excess of food reserve during late summer, the following spring finds the enzymes in a position to hydrolyze starches for immediate translocation and consumption. A number of variegated plants have been observed to blossom and fruit more quickly than is customary for the entirely green varieties.

Woods ('99) and Pantanelli ('05) considered that albinism in leaves is a constitutional disease in which the oxidizing enzymes, found always to occur more abundantly in the yellow plant parts than in the green, bring about a primary decomposition of the chlorophyll in certain portions and in certain cells of the leaves.

Pantanelli studied the distribution and the relative abundance of oxidases and peroxidases in the tissues of the variegated and green leaves of *Ulmus campestris* L., *Sambucus nigra* L., and *Acer Negundo* L. He confirmed the earlier results of Woods and further showed that in young leaves oxidases are more abundant than peroxidases, but that in old leaves the reverse relation holds. Pantanelli ('05) and Grandsire ('26) found cells in the chlorotic areas of variegated plants in a higher state of turgidity than in the green. The water content, too, of the light-colored areas is always higher.

In general, variegated varieties are more susceptible to mechanical injury, to fungous attacks, to extremes of temperature, and light intensity than are the green. The leaves, and the entire variegated plant as well, may be dwarfed and may remain sickly and undeveloped,—a further indication that in variegation and albinism we are dealing with a phenomenon which is, from the standpoint of the plant, a constitutional disease.

It is not always the case that the non-infectious types of variegation are inherited through the seed, although as a general rule this is true.

PHYSIOLOGY AND PATHOLOGY OF THE INFECTIOUS VARIEGATIONS

Though the "virus" theories proposed by Baur for infectious chlorosis ('06) have excited but casual interest, many of his experiments, and also those of Lindemuth, have been carried out with painstaking thoroughness. Since these papers have not been widely referred to in the modern literature on the mosaic diseases, it comes well within the scope of the present paper to review those which are especially outstanding.

Our knowledge concerning the nature of the disease and the physiology of infectious chlorotic plants is chiefly the result of the studies made by Baur upon species of *Abutilon*, *Cytisus*, *Evonymus*, *Fraxinus*, *Laburnum*, *Lavatera*, *Ligustrum*, *Ptelea*, and *Sorbus*. Lindemuth, on the other hand, confined his researches to a large number of genera, species, and varieties in the family Malvaceae. Our information in regard to the nature of immunity and susceptibility to infectious variegations among the members of this plant family may be chiefly ascribed to the investigations of Lindemuth. The salient contributions from all the researches in this field will be mentioned under the four major heads, which follow:

INFECTIOUS PROPERTIES OF THE CAUSAL AGENCY

Graft and tissue transplantation experiments.—Lindemuth ('78) and Baur ('04) have shown, as have other investigators before them, that the infectious chlorosis of certain species, particularly some of those belonging to the family Malvaceae, can be readily transmitted from an infected stock or scion either to the green

varieties of the same species or to related susceptible species, by grafting or by the transplantation of living leaf or stem tissue to the wounded surface of a growing stem. Obviously enough, in the latter case, where only living-leaf tissue is used, this transfer of infectious agency must pass quite rapidly out of the infected branch into the susceptible green stock. Careful experiments are still needed to determine the minimum of time which must elapse after a graft union has once been established in order to bring about the first visible evidence that a transfer of the virus[1] from stock to scion or vice versa has been made.

Rischkow ('27a) has given attention recently to the infectious chloroses of *Evonymus japonica*, one of which he described as having the appearance of light yellow stripes along the veins at an early stage in the development of the leaf. The infectious variegation, he states, is associated with the other types of variegations which are non-infectious, such as, for example, the foliage varieties "*marmor*," "*chlorino-marginata*," and "*aureo-maculata*," so that its identity is nearly or entirely masked by them. To establish the existence of the infectious variegation it was necessary for him to graft a plant of *Evonymus japonica* having variegated leaves with one having the uniformly green leaves, so that the chlorosis might be expressed free from all other types.

In all cases it was necessary for a period to elapse, varying in length from one to several months, following the union between stock and scion, before the chlorosis first appeared along the veins of the youngest green leaves. When the grafted parts failed to unite, in no case was the transmission of the variegation acquired. Ageing of the infected leaves was accompanied by a gradual disappearance of the chlorotic stripes along the veins, a change which was frequently accompanied by certain morphological changes which resulted in the formation of chlorotic

[1] The author is aware of no experiments which have been made which can be sufficiently substantiated to warrant the use of the term "virus" without some qualification when it is applied to the causal agency associated with infectious types of variegations. However, for want of a more correct term he is obliged frequently to use it in this paper when reference is made to the causal agency producing "infectious chlorosis." Hereafter the words "virus," "causal agency," and "infectious agency" will be used interchangeably.

areas among the mesophyll cells of the leaf. A criticism which may justly be applied to the conclusions presented in the paper of Rischkow is the fact that they have been drawn from experiments involving single plants.

Inoculation tests.—Baur ('04) made persistent attempts to transmit the variegation by applying juice from the crushed leaves of an infected plant to various species of Malvaceae, but his experiments were always without success. Lindemuth ('07) mentioned earlier unsuccessful attempts of his own and of Lewin to infect susceptible Malvaceae by injecting infecting juices of *A. Thompsonii* into the bark, and by watering the roots of potted plants with the juice from the variegated leaves.

Efforts to inoculate various species and highly susceptible green Malvaceae have been made by cutting and grinding variegated leaves into a pulp which was then pasted over extensive wounded surfaces. Sap has been pressed from spotted leaves and injected, filtered and unfiltered, into sound twigs which were cut off for the purpose and then grafted back upon the mother plants, or these twigs were grown as cuttings. Baur failed to give any statement as to the kind of filter which he used or the methods which were used in applying the filtering process. He completely immersed twigs in a vessel containing expressed juice of infected leaves, and then subjected the vessel to a reduced atmosphere of 20 mm. mercury by using a mercury air pump. The sap was allowed to flow back into the intercellular spaces under normal air pressure. Of the pieces that survived this treatment, some were rooted as cuttings while others were grafted back upon the parent stock. These experiments all gave negative results. To date no experiments have been recorded that show a transfer of the variegation except when two plant parts, one variegated (diseased), the other green (susceptible), are placed in close contact, so that the living cells of one are in intimate relation with the living cells of the other.

Attempts at transmission by insects.—Rischkow ('27b) conducted an experiment with a view to determining whether insects feeding on *Evonymus japonica* infected with chlorosis could transmit the causal agency to healthy plants. He kept the red spider, *Tetranychus telarius*, feeding and reproducing in large

numbers on the foliage of two plants placed adjacently, one infected with chlorosis, the other normal green. The experiment lasted for four months but no transfer of the infectious chlorosis was obtained.

The causal agency in relation to plant organs.—Various organs of the susceptible plants behaved differently as regards the infectious properties of the causal agency.

Root.—For the roots Baur ('04) wrote, "Ich verbrachte dann ferner ausgetopfte gesunde Pflanzen für Stunden und Tage mit ihren Würzeln in den unfiltrierten Presssaft aus kranken Blättern, alles mit demselben negativen Erfölge." However, Lindemuth ('07) obtained repeated infection through the root of *Althaea rosea* (L.) Cav. by grafting the entire plant high upon the stem of *Abutilon Thompsonii*. There were variegated leaves left below the graft. In this case there was established a union between the fleshy perennial root and the cambium of the stem.

Stem.—Baur concluded from certain observations from crude experiments that the virus travels very slowly through the phloem region of the stem. This conclusion is, however, not especially well founded as is obvious from the following description of his experiment. Ringing experiments were performed to determine whether or not the virus travels through the xylem or phloem regions of the stem. Plants of *A. avicennae* Gaertn. were ringed for a width of 0.5 cm. and a shoot of *A. Thompsonii* was grafted in some experiments below and in others above the ringing. The experiment was carried on three times, once with the graft above and twice with the graft below the girdling. In the first case, the plant *A. avicennae* was infected at the tip after an interval of two weeks. It developed three small stunted leaves and died four weeks after the operation. The stock below the ringing developed two dormant axillary buds before the death of the tops above the girdle. These buds produced two strong shoots during the course of the summer. Both shoots remained green-leaved. In the other two cases the grafted shoot of *A. Thompsonii* likewise grew well, living in union with the stock twelve weeks before the experiment ended.

As far as is known, the stem is not in any way visibly affected by the disease. If Baur is correct in his assumption that the

phloem alone carries the infectious ingredients, it would be of interest to study the vascular strands in stem and in leaf tissues for any structural modifications which might be the results of chlorosis infection.

Another point of interest in connection with a discussion of the infectious properties of the causal agent in relation to the stem has been pointed out by Baur. He reached the conclusion from experiments with certain species of *Abutilon* that the active agency can be carried from an infected stock through a healthy scion. In its turn the healthy scion may infect the healthy scion of a susceptible species grafted upon it without itself succumbing either to the infection from below, or, as would be the case later, from the infected scion above. The experiment will be described under the heading "immunity" which is to follow.

Leaves.—Depending upon the stage of development, leaves, as in the unexpanded buds or in the expanded leaf blades, appear to exhibit distinct peculiarities toward the infectious agency. A description of typical experiments will show these relationships as Baur considered them to be true.

On strongly variegated specimens of *Abutilon Thompsonii* Baur grafted scions of a green-leaved variety of *A. arboreum* which is susceptible to infectious chlorosis. On a portion of these the leaves on the *A. Thompsonii* stock were left attached, and on another portion the leaves on the stock were removed and no new leaves were allowed to develop. On the latter the scions all remained green, on the former they became variegated. Some time later, an axillary bud was allowed to give rise to a variegated shoot upon a stock of the previous experiment on which the leaves had been removed and the scion of which had consequently remained green-leaved. Three weeks after the first variegated leaves had appeared on this shoot, the scions developed variegated leaves.

Buds which are formed while the plant is variegated will develop later into variegated leafy shoots and will infect the plant even if, in the meantime, the plant has become completely green-leaved through proper light treatment. Buds which will later produce variegated leaves have no power to infect while

they are present in the dormant condition. Where buds from an infected plant are transplanted to a susceptible green-leaved plant, infection takes place more rapidly and in a larger percentage of cases where the variegated leaf of the bud is also transplanted.

Leaves which are in the process of expanding from the closed-bud stage are seen to require a period of development, or enlargement, before they can be observed to possess mottling either under reflected or transmitted light. At this stage they seem to be well supplied with chlorophyll.

Baur believed that he had proved by experiment that these light-colored spots are centers for both the reproduction and the infection of the virus. Along with the increase in size, a leaf which has become variegated can probably bring about infection in still younger leaves, so that the infection spreads, always in the vicinity of the actively growing tips. Leaves which are already mature and uniformly green when the infection spreads will remain so.

Seed and stem.—It seems to be a general observation that those variegations which have proved to be infectious chloroses are not usually transmitted through the seed from the infected parent stock. However, there are many examples which have not yet been tested adequately.

In this connection an experiment of Lindemuth ('07) may be mentioned. Of ten seedlings that grew from seeds of *Lavatera arborea* L. infected with *Abutilon Thompsonii* variegation, one became variegated at once, three became variegated later but eventually lost the variegation entirely, while six grew green-leaved from seed. In the case of the horticultural variety of *Abutilon*, "H. Cannell," the infected plant produced seed from which there were grown twenty seedlings, three being variegated. Lindemuth reported that further details connected with the ancestry of the seed were lacking. He was inclined to believe the variegated seedlings arose spontaneously.

Baur advanced the hypothesis that in developing leaves the virus accumulated, by drawing from the general supply in the stem, only when the leaves had developed so far as to provide for a material interchange of food products in the direction of

the stem and vice versa. Upon this assumption it is only necessary to argue that in the embryos of seeds the potential leaf organs have not yet reached this stage in their development, and that without an accumulation of the virus from the stem there is too little present in the seeds to bring about infection.

That the virus exists in such small amounts in the stem of a variegated plant has been assumed from the fact that a plant can be cured by removing all the leaves for two successive crops. The third crop of leaves to appear will be pure green. In other words, by this procedure it has been shown, according to Baur, that there is only a limited amount of virus located in the stems, and that this supply has been exhausted after the first two crops of leaves have been infected and subsequently removed.

Floral parts.—There are many observations to show that variegation also occurs in the tissues of floral parts. Some variegations have been known to occur on plants infected with chloroses and probably result from the infection, while others, such as the yellow stripes on fruits of apple and pear, occur on non-infected plants, a fact which makes their pathogenicity seem doubtful.

In certain infected Malvaceae the leaves, ovary (including the carpels), as well as the young bark, may all show variegation, as, for example, *Althaea rosea* (L.) Cav. infected with the virus from *Abutilon Thompsonii* fruiting with variegated seed pods.

RELATION TO SOME EXTERNAL FACTORS

The infectious character of this disease bears a very direct relation to the presence of light. Here again Baur's work remains our chief source of knowledge in regard to the relation between the variegated properties of the disease types and certain of the external environmental factors.

From the earliest attempts to investigate the nature of the disease, it was found that the mosaic pattern of some variegated leaves held a close relationship to the intensity of sunlight. Lindemuth was the first to observe that variegated Malvaceae hold their characteristic mottling only so long as they are given a sufficiently high intensity of sunlight.

Light intensity experiments.—An extreme case was that in

which it was claimed by Baur that shielding the old variegated leaves from light was all that was necessary to inhibit what might be either the production or the translocation of the virus so that all new leaves developing at the vegetative growing points became and remained pure green, whether they were exposed to full sunlight or not.

It was also reported that by keeping the newly developing leaves in darkness for a time, infection was not hindered. The findings of Lindemuth were confirmed, and it was shown that strongly variegated plants could be made to lose the variegated appearance if placed for a time in sunlight of low intensity, whether they were allowed to remain in deep shade or given a small fraction of the direct sunlight for only a portion of the day. Baur's experiments were, however, of the crudest sort. The yellow spots on the newly developing leaves were said to become smaller and sparser until the leaves showed isolated yellow flecks and in the course of time became pure green. In the younger leaves the greening proceeded most rapidly, while older leaves in which the variegation formed under the better source of light remained for a long time without noticeable change. Where the old leaves were removed the greening of the entire plant was said to go on more rapidly. This is, however, a difficult point to verify. From these facts the following conclusions have been reached. The quantity of virus formed in a spotted plant is dependent, first, on the light intensity, and second, on the size of the yellow spots in the leaves.

Quality of light.—To a certain extent, experiments with blue-green and red light gave similar results. The plants remained spotted in both glass houses but clearly less in the blue than in the red.

Inasmuch as the plants under this treatment were placed in a position where they remained in the shade for a half of the afternoon, it is doubtful whether the results which Baur obtained can be attributed to the quality of light. What makes his interpretation even more unlikely is the additional fact that the intensity of light would be materially reduced under colored glass and especially under blue glass. The experiment shows, however, that the mottled condition can be produced under light in both halves of the spectrum.

Carbon dioxide.—The question which Baur raised, "Is the virus production connected in some way with the carbon dioxide assimilation process?" remains unanswered. The obvious experiment of growing variegated plants for a long time in carbon dioxide-free air was tried, but failed because the experimental plants became defoliated after a few days.

Soil and mineral nutrients.—Future experiments will undoubtedly determine to what extent, if any, fertilizers and soil factors can be taken into consideration in the production or inhibition of the mottled condition of the leaves. This phase of the problem did not receive much attention by the earlier workers. A single statement by Lindemuth, to the effect that soils high in fertility did favor a strong infection may doubtless stimulate future investigations along this line.

Climatic factors.—Lindemuth has shown that several shrub-like Malvaceae, such as *Althaea rosea* (L.) Cav., when infected, remained so during the vegetative period but lost the variegation during the course of the winter rest period. Other shrubs, for example, *Kitaibelia vitifolia* Willd., were said to retain the infectious chlorosis over the winter and for the remainder of life, in the stalks, leaf buds, or basal green leaves.

HOST RELATIONS

The relation of host to the mosaic patterns in the leaves.—The manner in which the infectious chlorosis was expressed in the various species differed considerably. In *Abutilon arboreum* only large single yellow spots appeared to interrupt the uniformity of the green; in other species, for example, *A. Sellowianum* Reg., the surface of the leaf exhibited a mosaic type of combination of clear green, clear yellow, and yellow-green fields in all possible gradations. In still other species, for example, *A. indicum* (L.) Don, the whole leaf became yellow or white except for a few small green spots, and remained small and wrinkled.

In the foliage varieties of *Ligustrum vulgare* an infectious type had been isolated which was present and obscured by a non-infectious variegation. It was said that if care were taken contagious symptoms might be recognized on young leaves by the yellowing along the veins. There appeared only an incon-

spicuous yellowing of the leaves on species of *Laburnum* carrying the infection. The sharply defined variegated spots or stripes were missing. In the particular case of *Sorbus Aucuparia*, the infectious variegation was further modified so that only the tips of the teeth on the margin of green leaves were chlorotic-yellow.

Limits of susceptibility and of immunity to the virus.—Lindemuth ('78, '99a, '99b, '02a, '02b, '07) has given special attention to the extent to which different members of the Malvaceae are susceptible. His first paper appeared in Berlin in 1870. A very excellent review of all of his investigations and a comprehensive discussion of the literature is included in his 1907 paper. Different species of Malvaceae were shown to differ markedly in their ability to withstand and to take the infection. The author of the present paper has arranged the infectious species referred to in the literature in a table which accompanies this discussion. Footnotes refer to the relative degree of susceptibility or to the type of resistance found.

Abutilon indicum and *Sida Abutilon* show a type of variegation consisting of a single more or less expanded yellow spot in the leaf with little of the green remaining. Such plants are so severely infected that they frequently die because of the inhibited carbon-dioxide assimilation. According to Lindemuth ('07), resistance and immunity may depend upon the individual characteristics of the plant, upon the season, or upon the methods which are used in making the graft and transplantation. These points are illustrated in Lindemuth's experiments with *Abutilon arboreum*. He used *A. arboreum* four times as a scion upon *Abutilon Thompsonii* and twice as a stock for *A. Thompsonii* scions. Of this number, *A. arboreum* was infected three times as a scion and once as a stock. Old plants did not take the infection readily if at all. At times as many as twenty grafts were made between certain susceptible green and infectious chlorotic individuals, with the result that none became infected. At other times he was able to infect three out of every five plants. *Lavatera arborea* L. has been considered by some to be immune, yet Lindemuth was able to show, by using large numbers of individuals at different times, that its range of susceptibility varied all the way from immunity to a super-susceptibility.

Interesting experiments have been performed by Baur in this general connection. Scions of an immune strain of *A. arboreum* were grafted on several strongly variegated plants of *A. Thompsonii*. The scions remained pure green. On part of these *A. arboreum* scions were grafted shoots of highly susceptible *A. indicum* stock. Within a short time these superiorly placed scions of *A. indicum* became variegated, although the subsequently formed leaves of the *A. arboreum* scions never became yellow-spotted. The conclusion was drawn that the virus was carried up through the *A. arboreum* scion which was immune to it, and entered the topped-graft scion of *A. indicum* without becoming in any way inactivated in the course of the process. However, scions of *A. arboreum* which were held for a time in union with a variegated stock of *A. Thompsonii* and later removed and grafted upon a highly susceptible stock of *A. striatum* never succeeded in transmitting the variegation to the susceptible *A. striatum* stock. The assumption was made as a result of this experiment that the virus did not reproduce itself while in the latent condition in the stem of the immune *A. arboreum*.

In another instance, an immune strain of *Abutilon striatum* Dicks. was established through the spontaneous development of two pure green shoots on a strongly variegated plant of *A. striatum*. These were eventually removed and propagated as cuttings. All of these individuals remained immune to infectious chlorosis in spite of attempts to infect them by grafting the latter upon other chlorotic Malvaceae. It was not discovered whether this immunity carried itself through the seed or not.

Characteristic immunity in Malvaceous plants may be of three types, according to the assumptions of Baur: (1) The non-infected parts of plants may show immunity by preventing the passage of the virus into such parts. (2) After entering the previously non-infected parts the virus may be subsequently rendered inactive. (3) The virus may gain entrance into the non-infected parts, and then in some way it may be possible for the "infected" plant to remain indifferent to the virus without destroying the infectious character of the latter. On the other hand, Lindemuth chose to consider experimentally the

immunity for each individual before he would believe in any one type of immunity which could be applied to a whole group of plants.

Distribution of the causal agency among plants.—There have been reported within the last two hundred years large numbers of isolated cases where variegations have been said to result from stock and scion infection. However, few observations have met the approval of scientific investigators for the reason that they have been largely described by laymen who have little or no insight into other possibilities in connection with their appearance. With so many variegations in horticultural establishments which answer the general description of the infectious types, there is still a necessity for extensive and reliable studies to be made in spite of the extent to which the initial efforts of Baur and Lindemuth have contributed.

There are frequently found in recent horticultural literature references to new forms of variegated varieties appearing spontaneously outside of cultivation among the wild plants. It is exceedingly likely that in certain cases the causal agency of infectious chlorosis is responsible for the sudden appearance and the spread of these within the limits of localized areas.

Until some direct or indirect method can be found to carry the virus to unrelated plants other than by grafting, the investigator seeking to establish the communicability of infectious chlorosis to other species is seriously handicapped. Relatively few grafts are congenial when carried on outside of closely related varieties and neighboring species. Table I furnishes a fair approximation of the extent to which infectious chlorosis has been transferred between members belonging to the plant families Caprifoliaceae, Celastraceae, Cornaceae, Leguminosae, Malvaceae, Oleaceae, Rosaceae, and Rutaceae. Other families, the genera of which have been suggested as having possible relations to types of infectious chlorosis, are Euphorbiaceae and Nyctaginaceae.

Summarizing the information, the following points require emphasis: (1) The manner in which the infectious chlorosis is expressed in the various species differs considerably; thus, variegations may be recognized easily or with relative difficulty.

TABLE I

HOST RANGE OF INFECTIOUS CHLOROSIS

Source of the virus	Host recipient	Variegation transmitted through				Authority
		Graft	Bud	Tissue	Seed	
Abutilon striatum Dicks. var. *Thompsonii* Veitch.	*Abutilon* var. Africain	+				Lindemuth
A. striatum Dicks. var. *Thompsonii* Veitch.	*A. arboreum* Sweet*	±	−		−	Baur, Lindemuth, Morren
A. striatum Dicks. var. *Thompsonii* Veitch.	*A.* var. *atrosanguineum*	+				Lindemuth
A. striatum Dicks. var. *Thompsonii* Veitch.	*A. Avicennae* Gaertn	++	−		−	Baur, Lindemuth
A. striatum Dicks. var. *Thompsonii* Veitch.	*A.* var. Cannell	+				Lindemuth
A. striatum Dicks. var. *Thompsonii* Veitch.	*A. Darwinii* Hook	+				Lindemuth
A. striatum Dicks. var. *Thompsonii* Veitch.	*A. Darwinii* var. *tessellatum* Hort.	+				Lindemuth
A. striatum Dicks. var. *Thompsonii* Veitch.	*A.* var. Erfurter Glocke	+				Lindemuth
A. striatum Dicks. var. *Thompsonii* Veitch.	*A. esculentum* St. Hil	+				Lindemuth
A. striatum Dicks. var. *Thompsonii* Veitch.	*A.* var. Feuerball	+				Lindemuth
A. striatum Dicks. var. *Thompsonii* Veitch.	*A.* var. Firefly	+				Lindemuth
A. striatum Dicks. var. *Thompsonii* Veitch.	*A.* var. Wilh. Heuer	+				Lindemuth
A. striatum Dicks. var. *Thompsonii* Veitch.	*A. hybridum* var. *grandiflorum* Hort.	+				Lindemuth
A. striatum Dicks. var. *Thompsonii* Veitch.	*A. inaequale*	+				Lindemuth
A. striatum Dicks. var. *Thompsonii* Veitch.	*A. indicum* Sweet	++	−		−	Baur, Lindemuth
A. striatum Dicks. var. *Thompsonii* Veitch.	*A. insigne* Planch	±	−			Morren, Lindemuth
A. striatum Dicks. var. *Thompsonii* Veitch.	*A.* var. Karl Schweizer	+				Lindemuth
A. striatum Dicks. var. *Thompsonii* Veitch.	*A.* var. Lemoine	+				Lindemuth
A. striatum Dicks. var. *Thompsonii* Veitch.	*A.* var. Martins	+				Lindemuth
A. striatum Dicks. var. *Thompsonii* Veitch.	*A. megapotamicum* St. Hil. & Naud	+	−		−	Baur, Lindemuth, Morren
A. striatum Dicks. var. *Thompsonii* Veitch.	*A.* var. *Patersoni*	+				Lindemuth
A. striatum Dicks. var. *Thompsonii* Veitch.	*A.* var. Reve d'Or	+				Lindemuth
A. striatum Dicks. var. *Thompsonii* Veitch.	*A.* var. Royal Scarlet	+				Lindemuth
A. striatum Dicks. var. *Thompsonii* Veitch.	*A.* var. *Savitzii* (tricolor)	+				Lindemuth
A. striatum Dicks. var. *Thompsonii* Veitch.	*A.* var. Schneerose	+				Lindemuth
A. striatum Dicks. var. *Thompsonii* Veitch.	*A. Sellowianum* Regel	++	−		−	Baur, Lindemuth

TABLE I—*Continued*

Source of the virus	Host recipient	Variegation transmitted through				Authority
		Graft	Bud	Tissue	Seed	
A. striatum Dicks. var. *Thompsonii* Veitch.	*A.* var. Souvenir d'Arago	+				Lindemuth
A. striatum Dicks. var. *Thompsonii* Veitch.	*A.* var. Souvenir de Bonn (tricolor)	+				Lindemuth
A. striatum Dicks. var. *Thompsonii* Veitch.	*A.* var. Souvenir de Kotschyi Hochst	+				Lindemuth
A. striatum Dicks. var. *Thompsonii* Veitch.	*A.* var. spec. 234	+				Lindemuth
A. striatum Dicks. var. *Thompsonii* Veitch.	*A.* var. *striatiflorum*	+				Lindemuth
A. striatum Dicks. var. *Thompsonii* Veitch.	*A. striatum* Dicks.	++	−		−	Baur, Lindemuth, Morren
A. striatum Dicks. var. *Thompsonii* Veitch.	*A.* var. *sulphureum*	+				Lindemuth
A. striatum Dicks. var. *Thompsonii* Veitch.	*A. venosum* Lem.	+	−		−	Baur, Lindemuth, Morren
A. striatum Dicks. var. *Thompsonii* Veitch.	*A. vitifolium* Presl	+				Lindemuth
A. striatum Dicks. var. *Thompsonii* Veitch.	*A.* var. Philippine Welser	+				Lindemuth
A. striatum Dicks. var. *Thompsonii* Veitch.	*Althaea ficifolia* Cav	+				Lindemuth
A. striatum Dicks. var. *Thompsonii* Veitch.	*A. officinalis* L.	+				Lindemuth
A. striatum Dicks. var. *Thompsonii* Veitch.	*A. rosea* Cav.	+	−		−	Baur
A. striatum Dicks. var. *Thompsonii* Veitch.	*Kitaibelia vitifolia* Willd.**	±	−		−	Baur, Lindemuth
A. striatum Dicks. var. *Thompsonii* Veitch.	*Lavatera arborea* L.	±	−		−	Baur, Lindemuth
A. striatum Dicks. var. *Thompsonii* Veitch.	*Malva sylvestris* L. var. *mauritiana* Mill.	+				Lindemuth
A. striatum Dicks. var. *Thompsonii* Veitch.	*M. verticillata* L.	+				Lindemuth
A. striatum Dicks. var. *Thompsonii* Veitch.	*Malvastrum capense* Garcke	+				Lindemuth
A. striatum Dicks. var. *Thompsonii* Veitch.	*Modiola decumbens* G. Don	+				Lindemuth
A. striatum Dicks. var. *Thompsonii* Veitch.	*Sida mollis* Herb.	+				Lindemuth
A. striatum Dicks. var. *Thompsonii* Veitch.	S. *Napaea* Cav	+				Lindemuth
A. striatum Dicks. var. *Thompsonii* Veitch.	*Sidalcea candida* A. Gray	+				Lindemuth
A. striatum Dicks. var. *Thompsonii* Veitch.	*Anoda hastata* Cav	+			−	Lindemuth
Cornus alba L.	On others	−				
Cornus alba var. *argenteo variegatum elegans* Hort.	*Cornus alba*	−				Baur, others
Evonymus japonica L. var. *argenteo marginatus*	*Evonymus japonica*	−			−	Baur, Bouché
E. japonica L. var. *aureo-marginata* Hort.	*Evonymus japonica*	+				Baur

TABLE I—*Continued*

Source of the virus	Host recipient	Variegation transmitted through				Authority
		Graft	Bud	Tissue	Seed	
E. japonica var. *marmor*	*Evonymus japonica* var. *chlorino-marginata*	+				Rischkow
E. japonica var. *marmor*	*E. japonica* var. *aureo-maculata*	+				Rischkow
E. japonica var. *marmor*	*E. radicans* var. *marmor*	+				Rischkow
Fraxinus pubescens Lam. var. *aucubaefolia* Hort.	*Fraxinus* spp.	+				Baur
Jasminum officinale L. var. *variegata* Hort.	*Jasminum revolutum* Sims		+			Masters, Morren
J. revolutum Sims. var. *aureo-variegata*	*Jasminum officinale*	+				Masters, Morren
Laburnum vulgare Griseb. var. *chrysophyllum* Spath.	*Laburnum alpinum* Griseb.	−				Baur
L. vulgare Griseb. var. *chrysophyllum* Spath.	*Cytisus hirsutus* var. *chrysophyllum* Reiser		+			Baur
L. vulgare Griseb. var. *chrysophyllum* Spath.	*C.* var. *purpurens*	−				Baur
L. vulgare Griseb. var. *chrysophyllum* Spath.	*Laburnum vulgare* L.	+				Baur
L. vulgare var. *aurens* Hort.	*Laburnum vulgare** ‡	+	+	+		Baur
Ligustrum vulgare L. var. *aureum* Hort.	*Ligustrum vulgare*	−				Baur
L. vulgare var. *aureo-variegatum* Spath.	*Ligustrum vulgare*	+	+		−	Baur
L. vulgare glaucum var. *albo-marginatum* Spath.	*L. vulgare*	−			+	Baur
L. vulgare var. *aureo-variegatum* Hort.	*L. vulgare* var. *aureum*	+			±	Baur
Ptelea trifoliata L. var. *aurea* Hort.	*Ptelea trifoliata*	−	−	−	+	Baur
P. trifoliata var. *variegatum*	*P. trifoliata*	+				Baur
Sorbus Aucuparia L. var. *Dirkenii aurea*	*Sorbus Aucuparia*	−				Baur
S. Aucuparia L. var. *lenteo-variegatum*	*S. Aucuparia*		+			Baur

* An immunity which permits the virus to pass through the stem with unaffected infectious properties.
** An immunity specific with certain individual specimens.
\+ In all cases transmissible.
− In no case transmissible.
± Transmissible in some instances, not so in others.
++ Very strongly susceptible.
‡ Susceptible to the transplantation of cortex tissue from the infected stem.

(2) Infectious chlorotic variegations are usually of the typical *aurea* (yellow) type. (3) Strongly variegated plants may send out green shoots which appear immune to infection. (4) Immune stems are said to carry the disease unaltered while the exact nature of individual immunity and susceptibility remains unknown. (5) A "variegation" may sometimes consist of an infectious chlorotic type in combination with a non-infectious type, in which case the former may be completely masked by the presence of the latter. (6) Infectious chlorosis is known to occur among eight plant families, eighteen genera, and roughly among thirty-five species.

NATURE OF THE CAUSAL AGENCY (A TRANSMISSIBLE TOXIN THEORY)

As a result of his studies, Baur ('06) postulated a toxin "virus" theory and stoutly opposed every suggestion that the infectious agency might be associated with a living entity of submicroscopical size. Nor did he concede the probability that species of *Abutilon* in tropical habitats become regularly infected with the infectious chlorosis agency through an intermediate and native insect carrier. He believed it more likely that the observed spontaneous spread of the variegation in the East Indies was due rather to its propagation by the inhabitants for ornamental purposes.

It is difficult to see how a critical examination of the evidence used by Baur to advance a toxin "virus" theory will permit any one to speculate very directly on the probable nature of the infectious agency. Certainly, in view of the modern knowledge and opinions in regard to the transmissibility and physiology of virus diseases of both plants and animals, such facts as those enumerated below appear entirely inadequate. Baur ('06b) has presented these facts in the support of the assumption that the causal agency for infectious chlorosis is not an organism.

> "Ich will trotzdem auf einige Ergebnisse der eben geschilderten Versuche kurz hinweisen, die mit der Annahme eines parasitären Organismus nicht gut vereinbar sind. Hierher gehört zunächst die absolute Abhängigkeit der Infizierung vom Lichte . . . Ferner die Tatsache, dass das Virus durch den Transpirationsstrom nicht geleitet wird, sondern, wie die Ringelungsversuche wenigstens sehr wahrscheinlich machen, nur in den Geweben, die der Leitung der plastischen Stoffe dienen. Drittens endlich der Umstand, dass das Virus, bei der Entstehung infizierter Blätter verbraucht, gebunden wird. Aus den

Versuchen folgt ja dass das Virus, das zu einem gewissen Zeitpunkte in einer Pflanze vorhanden ist, sich in den in einem gewissen Entwickelungsstadium befindlichen Blättern restlos ansammelt und hier festgelegt wird."

It is said that Beijerinck ('99), who saw the exhibit of Lindemuth's grafted Malvaceae, considered the chloroses in the same class of infectious diseases as the mosaic of tobacco. Baur ('06) has referred to the virus of tobacco mosaic as perhaps being more stable a virus than the "virus" of *Abutilon* chlorosis. To him the causal agency appears as a highly organized chemical substance which behaves as a toxin; differing, however, from other toxins in that it is synthesized directly in the infected cells after the manner of an autocatalytic chemical reaction. Lindemuth ('07), however, said little in regard to the nature of the infective agency but preferred to look upon it as possessing life and the power of reproduction.

ANATOMY OF LEAF VARIEGATIONS

The recent investigations of Funaoka ('24), Tsinen ('23, '24), Hein ('26), Smith ('26), and Küster ('27) have materially advanced our knowledge upon the anatomy, histology, cytology, and pathology of non-contagious variegations. The paper by Küster referred to above is a monographic treatment of the literature and cites 159 references of which 42 have been published during the last five years. It is not intended here to do more than reiterate a few of the outstanding facts in regard to the anatomy of variegations.

Küster ('27) arbitrarily divided all variegations into two classes: variegations in which the limitation of the chlorotic areas appear irregular, and variegations with sharply limited chlorotic areas. He stated that it is to the former class that all or most pathological or infectious variegations belong.

Smith ('26) has observed in the chlorotic mesophyll cells of living and fixed sections of *Evonymus japonica* vars. "*mediopicta*" and "*argenteo-variegata,*" "vacuolate bodies comparable to those found in tobacco and petunia mosaics." However, it is an error to state that both of these varieties were studied by Baur. To the knowledge of the present writer the former of these variegations has not been mentioned in any of Baur's

work. While the "*argenteo-variegata*" type is mentioned, it is probable, from Baur's remarks, that it is not to be considered infectious:

> "Die erstere Varietät ist nicht infektiös, ich habe eine grosse Anzahl von Pfropfungen von grünen auf weissrandige und von weissrandigen auf grüne Pflanzen mehrere Jahre hindurch beobachtet."

These recent observations by Smith appear significant when it is remembered that the occurrence of vacuolate bodies is being used by some plant pathologists and plant cytologists working on the nature of the mosaic diseases as a criterion for the presence of the infectious virus. Should it become a well-established fact that such intracellular bodies are widely distributed among variegated phenomena, the present interpretation of their presence in the cells of tobacco plants affected with the mosaic disease will need revision. In the meantime every effort should be made to determine whether a contagious variegation exists in these varieties of *Evonymus*. The reader is referred to the recent publication of Rischkow ('27b) and to the author's experiments which are described in this paper as having some significance in this connection.

As a possible explanation for the occurrence of vacuolate bodies in variegated plants where the transmissibility of variegations has been doubted, is the view, taken by some, that the presence of a striking leaf pattern of a non-infectious type of variegation may obscure the existence of a faintly expressed type of infectious variegation associated with the former in the foliage leaves. It has been shown, in the case of *Evonymus japonica* and *Ligustrum vulgare*, that in some instances where the infectious and the non-infectious types occur together, they can be separated by grafting the variegated upon the green variety. By this procedure the transmissible type may then infect and cause the variegation to appear in the tissues of the green leaves. The possibility exists that if such an infection could be brought about experimentally in the case of *Evonymus japonica* vars. "*mediopicta*" and "*argenteo-variegata*," there might be found in the mesophyll cells of the infected plants vacuolate bodies comparable to those already found by Miss Smith. Such a discovery would justify her conclusion, namely, "the vacuolate bodies, therefore,

have been observed as yet only associated with the infectious chloroses." Until work is done which proves that the material described by Smith contained an infectious variegation associated with the non-infectious varieties "*medio-picta*" and "*argenteo-variegata*," it can be concluded that vacuolate bodies typical of those found in cells infected with mosaic disease of tobacco have been found in the chlorotic cells of a variegation which is infected with neither infectious chlorosis nor mosaic diseases.

As a result of his cytological studies upon *Evonymus japonica* varieties infected with chloroses, Rischkow ('27b), who was plainly unaware of the findings of Miss Smith, has reached the conclusion that the "infectious chlorosis" and the mosaic diseases can be distinguished from each other chiefly by the absence of the X bodies from the cells of leaves infected with chlorosis. The data from his cytological studies can be criticized, first, because the samples were taken from single plants, and second, that a statement of the cytological methods which were used is not included. Since it is well known that X bodies found in the leaves of tobacco diseased with mosaic are soluble in some killing fluids while insoluble in others, it is difficult to understand why Rischkow should have neglected to mention the killing fluid which was used in the preparation of his stained sections.

One of the chief contributions made by Rischkow on the cytology of infectious chlorosis in *Evonymus* results from the fact that he describes transparent pustules which arise upon the under side of the leaves and project from the lower surface for a distance not greater than 90–100 μ. The author states that large numbers of these pustules are present when a plant is infected with contagious variegation, although very few if any can be found upon the leaves of a healthy plant. By sectioning, it can be shown that they originate through the hypertrophy of groups of single cells in the mesophyll. Typically, the intumescences are enlarged cells rich in water and with more or less decolorized chloroplasts. As the leaves of normal plants change from green to chlorotic as the result of infectious grafts it is said that the number of intumescences is increased along the lateral veins of young leaves at a rate which permits them to keep abreast the development of the chlorotic stripes.

Bouché ('71) observed a variegation, proved by him to be infectious upon *Evonymus japonica*, which could be seen only with difficulty in old leaves but more easily in young leaves. He believed this was because it followed only the conductive strands of the leaf. Although Rischkow ('27b) failed to mention the work of Bouché, it would appear probable from the descriptions of his variegation that his "geadertepanaschierung" is the same type of infectious variegation as that of Bouché.

For the infectious Malvaceae, Küster touched briefly upon the fact that the richly sprinkled pale and green areas are polygonal, for the reason that the conductive strands of the netted system very frequently become the limits of the pale and green fields. Differentiations of the tissues and cells were discussed. In cross-sections of some tissues the thickness of the pale portion was the same as the green. In others there were striking differences which expressed the inhibition of tissue formation in the light-colored leaf areas.

The cytology of plants infected with this type of chlorosis needs to be worked out carefully for various environmental conditions. The bleaching and the metamorphosis of chloroplasts need to be studied in connection with environmental influences. The true nature of the vacuolate bodies of Smith ('26) needs also to be studied.

Hein ('26) and Smith ('26) described the degeneration of chloroplasts in living and fixed material for a number of non-infectious leaf variegations.

Chemistry of Variegations

Pigments

Aside from the obvious deficiency of chlorophyll pigments in the pale areas of all variegated leaves, it is of interest to note that in general, and possibly with but very few known exceptions, the virus causes a yellow instead of a white, brown, red, or other colored leaf character to appear. Whether a greater production of carotinoid pigments accompanies the infectious disease has not as yet been satisfactorily determined. It may be that the yellow color will be simply explained by the reduction in the amount of chlorophyll so that the other leaf pigments are no longer masked by the dark green pigment.

The concentrations and the relationships of flavones and anthocyanin pigments to variegated species have received no attention.

It would be of interest to know whether the normal ratio of chlorophyll a/chlorophyll b and of carotin/xanthophyll remains the same in infected varieties as they are in the green varieties. In the non-infectious variegated *Coleus*, var. "Golden Bedder," Schertz ('21) found great deficiency of a and b chlorophylls and more xanthophyll and more carotin than in the green.

CARBON DIOXIDE ASSIMILATION PRODUCTS

Schertz ('21) found starch present, especially in the guard cells of mottled leaves and other places in the chlorotic area, sufficient to give a good test with iodine. Studies on the total carbohydrate content and its periodic fluctuations showed that photosynthetic activity in the mottled leaves was greatly reduced. Grandsire ('26) has shown that in the albino leaves of *Hemerocallis*, *Acer*, *Cornus*, and *Spiraea* there is as great a variety of carbohydrates as there is in the green, but they are notably lower in absolute amounts. Starch, however, is regularly absent.

NUTRIENT RELATIONS: ASH, INORGANIC SALTS

All investigators seem agreed that there is a higher ash content in the variegated portions of leaves than in the green. In organic matter the opposite is true. From his analyses, Grandsire ('26) showed that albino leaves of the genera mentioned were poor in calcium and rich in potassium. Phosphorus was present in equal proportions in the green and white leaves at the beginning of growth but diminished with age, a condition which was more marked in the white. The ash of white leaves was distinguished from the green in that it had a higher percentage of soluble phosphorous pentoxide. The quantities of other ash constituents, such as iron, magnesium, and sulphur, differed only slightly in amount.

METABOLIC ACTIVITY

Osmotic pressure.—It is generally recognized that pale areas and albino leaves are higher in water content, as well as in the osmotic pressures of the cells. Pantanelli ('05) has made the

suggestion that the latter fact may be due to the presence of metabolic constituents of low molecular weight.

Nitrogen.—Variegated leaves are especially characterized by richness in soluble nitrogen and by a deficiency in total and nitrate nitrogen (Schertz, '21, and Grandsire, '26). Grandsire reported a steadily diminishing supply of total nitrogen, in both the green and in the albino leaves which he studied, as growth continued. However, total nitrogen losses in the variegated leaves were much more rapid than in the green. Insoluble nitrogen, whether referred to by dry or fresh weight, was always more abundant in the green than in the white.

Acidity.—The most painstaking studies have been made by Grandsire ('26), who obtained a low value for the total free or titratable acid content of pale areas in variegated leaves as compared to the green. Comparing the ash from chlorotic and green leaves with respect to combined acids (organic acid salts), the conclusion is reached that although the former leaves are higher in regard to total ash content they are actually lower with respect to combined acidity.

For the same samples this author found the ash of white leaves richer in soluble bases from the beginning to the end of the period of growth; the proportion of these bases increasing in albino leaves and diminishing in green leaves as growth continued. No electrometric titrations for total acidity or determinations for free hydrogen ions were made.

Presentation of Data

In addition to the work which was carried on at the Missouri Botanical Garden, a series of experiments was arranged at the Boyce Thompson Institute for Plant Research, at Yonkers, New York. It was hoped that they might lead to a better understanding of the nature of some of the effects which have been shown to occur when variegated foliage varieties have been placed under varying conditions of light and darkness and their leaf juices inoculated into healthy green varieties. Since some of the varieties used in the course of the experiments have been shown to be infectious variegations by Lindemuth, Baur, and others, interest in their behavior is stimulated by the fact that

after so many years so little is known in regard to the nature and cause of these diseases.

It has long been felt in the graduate laboratories at the Missouri Botanical Garden that this whole question should be reopened for further investigation with a view to relating the new and additional information to the general field of the mosaic and similarly classified diseases of plants. It was with such a purpose in mind that the following series of experiments was started. The first experiments to be described are physiological studies which have to do primarily with the influences of quality of light, intensity of light, and of darkness, all of which were considered by Baur in his researches upon infectious chlorosis.

THE EFFECT OF ENVIRONMENTAL FACTORS ON VARIEGATED LEAVES

EFFECT OF THE QUALITY OF LIGHT UPON THE PATTERN OF VARIEGATED LEAVES

Physical equipment.—The investigations were carried out in a range of five small adjacent greenhouses. These are referred to collectively as the spectral glass houses, inasmuch as they are equipped with sashes of different glasses especially selected for their ability to transmit certain wave lengths of the solar radiations and for holding back others. Each house was separately humidified and controlled with respect to temperature. The following table shows the glass used for each house and the wave lengths that it transmits.

TABLE II

WAVE LENGTHS INCLUDED IN THE VARIOUS VISIBLE AND ULTRA-VIOLET REGIONS AND SPECTRAL LIMITS OF GLASSES USED

Regions and glasses	Range in μ μ
Sunlight	290–720
Ultra-violet of sunlight	290–400
Violet	400–450
Blue	450–490
Green	490–535
Yellow	560–590
Orange	590–645
Red	645–720
Ordinary greenhouse glass (house I)	312–720
G 980 A. Corning (house II)	290–720
Noviol "O" Corning (house III)	389–720
Noviol "C" Corning (house IV)	472–720
G 34 (house V)	529–720

The relative intensity transmission was reduced in houses I

and II by means of shading inside with cotton netting so that these intensities would compare with those in the houses with limited spectra. On a very clear day the intensities in the different houses were measured with a Macbeth Illuminometer between 10:00 A.M. and 11:00 A.M., and they were each found to be approximately 3372 foot candles. Since the intensity of daylight varies from hour to hour and from day to day, no attempt was made to keep a constant record of it by measurements with a Macbeth Illuminometer or a pyroheliometer.

Plant material.—The varieties used in the first experiment were as follows: *Abutilon* var. *Savitzii; Evonymus japonica* L., varieties green, "*medio-picta,*" "*aureo-variegata,*" and "*argenteo-variegata;*" and *Pereskia aculeata* Mill., var. *Godseffiana,* and green. Both the green and the variegated varieties were placed in duplicate in each of the houses on September 24, 1926.

Results.—After two months under these conditions, the plants had produced an abundance of new growth. There seemed to be no observable correlations between the size, shape, or color of the light-pigmented leaf areas and the specific wave lengths transmitted in each of the houses. Except for an elongation of the internodes in the plants in houses IV and V, no striking changes could be observed in the general appearance of the plants nor of the foliage in one house as compared with another.

Material of *Abutilon Thompsonii* was available for use in late November, 1926, and ten plants were placed in each of the houses II, IV, and V, where they were left until the experiment ended on May 1, 1927. During the intervening months, variegated plants of the variety *Abutilon Thompsonii* under Corning glass G 980A in house II remained strongly variegated. The full intensity of the sunlight was permitted to enter the house.

Under the glass of both house IV (which excluded half of the blue rays) and house V (which excluded half of the green rays) all of the plants produced a very luxuriant growth. The variegated pattern of these plants changed noticeably from month to month during the winter and spring seasons. While most of the plants under normal greenhouse conditions retained the characteristic mosaic pattern without conspicuous modification, all of the plants in houses IV and V became less strongly varie-

gated. The yellow color in the chlorotic spots became less apparent in the older leaves, while in the newly developed leaves the spots were reduced in area as well as in the intensity of the yellow color.

To any one not already familiar with the extent to which the intensity and the duration of light may affect the variegation in leaves of *Abutilon Thompsonii*, it would appear reasonable to suppose that the quality of the light was the chief factor contributing to the condition of the chlorotic spots as described above. However, the author is disposed to consider the results as being brought about primarily by the shade which resulted from the nature of the glass used in houses IV and V. In all probability the short length of the winter days was a factor supplementing shade. It must be considered that each of these factors operated simultaneously in houses IV and V. In view of this fact, together with certain results obtained by the author showing the importance of the duration of the exposure to sunlight, the conclusion may be reached that a combination of the two factors, diminished intensity of sunlight and short day length, may account for the changed appearance of the variegated leaves.

EFFECTS OF CONTINUOUS ILLUMINATION

The experiments began on January 29 and officially ended, after a continuous run, on March 29. During this time the plants under the various conditions were examined daily, and extensive notes were taken describing their general appearance and especially the size and extent of the variegation developing in new leaves.

Physical Equipment.—The physical apparatus used in the control of the environmental factors for these experiments will be briefly described. The experiments were carried out under two sets of conditions, both available for use at the Boyce Thompson Institute. In one instance a gantry crane carrying forty-eight 1000-watt gas-filled lamps was used to supplement the sunlight of the day and was brought into action during the twelve hours of the night, being swung over the greenhouse shortly before sunset. The lamps were allowed to burn until shortly after sunrise. In the other instance a room humidified

and controlled with respect to temperature was lighted by means of twenty-five 1500-watt gas-filled lamps and three carbon arc lights suspended above a glass ceiling, which was kept flooded with water to eliminate direct heat rays. The lamps in this room burned steadily for twenty-four hours daily throughout the duration of the experiment. The plants were placed on a bench which circumscribed the room on three sides. The chief difference between the two conditions lies in the fact that plants were grown in the light room entirely under artificial illumination, while plants under the former condition received the daily sunlight and twelve hours of artificial light each twenty-four hours. The normal carbon-dioxide content of the air in both cases was supplemented by additional carbon dioxide from tanks under pressure. A self-recording electrical device was used to register variation in the concentration of the carbon dioxide in the vicinity of the plants under experiment, and meters were regulated to supply the gas uniformly. A house adjacent to the one receiving twenty-four hours of illumination accommodated all the control plants. This house was kept at a temperature of 78° F. with no extra light or additional carbon-dioxide gas. In a complementary experiment a small additional compartment under a roof of greenhouse glass was equipped with one 1000-watt electric lamp and a reflector. This was suspended above the tops of the plants at a height of two feet. All plants in this experiment received sunlight during the day and artificial illumination during the night.

Plant materials and methods.—Well-rooted cuttings of *Abutilon striatum* var. *Thompsonii* (pl. 6, fig. 1) and stout stock plants of the two *Evonymus japonica* varieties (pl. 6, fig. 3) showing new growth were placed under the conditions described above. Two entirely green plants of *Abutilon hybridum* with several green plants of *Evonymus japonica* were used as check plants under each treatment.

Representative plants from each condition were photographed on January 29 and were occasionally photographed thereafter until the end of the experiment two months later. Observations were frequently made. Samples were cut from chlorotic, green, and transitional areas of the leaves each week and then fixed in each of two killing fluids preparatory to embedding in paraffin.

Preliminary observations upon this cytological material will receive brief treatment under a separate heading. A presentation of cytological data in detail for the infectious variegations will appear soon in a subsequent paper.

Observations and conclusions.—The varieties of *Evonymus japonica* exhibited no visible or well-defined response to the conditions under which they were held while receiving continuous illumination, except for increases in stem growth. However, all plants of *Abutilon* showed an immediate response. All *Abutilon Thompsonii* held in the control greenhouse, 78° F., receiving no extra light and no extra carbon-dioxide gas, showed mottling only after the newly formed leaves reached a certain stage of development. Chlorotic areas never appeared before the new leaves were completely unrolled, but always before the leaves had been expanded for more than three to five days. The leaves at this stage enlarged exceedingly rapidly under all conditions and were capable of increasing in size from 16 sq. cm. to 64 sq. cm. in sixty hours. They may not have been visibly infected at the former size but they were in every case at the latter stage.

There seemed to appear first on these leaves very minute, lighter-colored islands, or flecks, which soon enlarged and coalesced with other flecks which were subsequently formed in the immediate neighborhood. An irregular, light-colored speck of a millimeter or so in diameter was produced by this fusion. It could be noticed that the first chlorotic flecks appeared frequently in the islands of parenchymatous leaf tissue which were in contact with the very finest tracheid endings. After a few days the leaves showed larger chlorotic areas, the size of fine bird shot, which were easily distinguished from the normal color. Later these yellow patches expanded to form still larger areas until they finally became limited by the larger vascular elements. From the course of the usual development, it can be said that the larger veins formed the boundary between the dark green and the lighter chlorotic areas. Typically mottled leaves of *Abutilon Thompsonii* under ordinary greenhouse conditions are shown in pl. 6, fig. 2. Just what factors in the leaves contribute to the production of such a uniformly mottled condition can not be explained at present.

Continuous 24-hour illumination.—Under the continuous 24-hour day in the light room, 78° F., with extra carbon-dioxide gas, with a battery of electric lamps and three carbon arcs to intensify the blue light, new leaves of *Abutilon Thompsonii* formed during the first ten days of the experiment became dark green, and so uniformly green, that unless they were examined under transmitted light they could not be distinguished from the leaves of normally green plants. A photograph showing these effects is given in pl. 7, fig. 1. In this paper the term "first-ranked leaf" will refer to the youngest expanded leaf at the growing tip; the "second-ranked leaf," to the next oldest leaf, etc. The upper and lower right-hand pairs of leaves in this photograph are taken from plants subjected to the same treatment and show the effect of the treatment on the variegation in different plants. In subsequent leaves the chlorosis developed as if its initial intensity had not been diminished.

As the experiment continued, mature leaves of the control green variety and all of the variegated plants of *Abutilon* became pale from an injury which may possibly be related to the quality of the light, its intensity, or perhaps to the absence of any period of rest. Chlorophyll was destroyed and in the case of the variegated *A. Thompsonii* the green areas disappeared temporarily, thereby leaving the leaves uniformly pale yellow in color. Photographs by transmitted light showed that the leaves possessed a mottling which had been masked by the acquisition of an additional chlorosis under the environment. New leaves of the variegated plants showed the infectious chlorosis as soon as plants in the control house, and in some cases sooner. The plants under continuous illumination were returned to the conditions in the control greenhouse at the completion of the experiments on March 29. Under these conditions new leaves developed free from the acquired chlorosis and mature leaves became plainly mottled.

Under the illumination of normal daylight together with the artificial light from a gantry crane, the condition of the plants under observation appeared to be essentially the same as that for the plants under continuous artificial light alone. *Abutilon Thompsonii*, however, did not show any decrease in the intensity

of the infectious chlorosis during the first ten days of the experiment, as was evident in the case of continuous artificial illumination.

EFFECT OF SHORT-DAY ILLUMINATION

Plants receiving light for five, seven or twelve hours in the continuous-light room were then shifted, according to the schedule, into a dark room where they were held at the same temperature and humidity without receiving light or extra carbon dioxide.

Five- and seven-hour day.—The conditions for growth appeared to be excellent for *Abutilon* but in the mature and new leaves the green areas developed somewhat less chlorophyll than the control plants. As the experiment continued, changes were apparent from week to week in the extent to which the chlorotic areas developed in newly formed leaves. Measurements were taken of the leaves when they first showed flecks, and these were compared with similar measurements taken in the control house and under the continuous-illumination experiments. The results suggest that although the first appearance of the infection may have been delayed to a certain extent, the chief difference lies in the rate at which the chlorotic areas enlarged, fused, and produced the mosaic pattern. As frequently observed under the five- and seven-hour-day length, the flecks first became visible by transmitted light when the leaves had an area of about 16 sq. cm. Upon developing an area of 61 sq. cm. it may be supposed, from the observations made upon the control and continuously illuminated plants, that the leaves should have a strong and well-defined mosaic pattern. However, this was not true in the latter case. For a few weeks after the new leaves reached this size under the shorter day lengths they still showed only traces of mottling. The axis of the main stem elongated much less rapidly under these short-day treatments than under the usual greenhouse conditions and the continuously illuminated environment.

On the other hand, where plants of Abutilon were grown under identically the same conditions except for twelve hours of artificial day and twelve hours of total darkness, the foliage remained brightly variegated, without change until near the end of

the experiments when some of the intensity of the chlorotic condition was lost. The fact that they then commenced to lose the variegation and to become more uniformly green may have been due to the slight fall in the intensity of the light in the continuous-light room, a condition which was observed during the last two weeks of the experiment.

Samples were taken and fixed for staining during the course of each week from one-half of the plants under each condition and from check plants held in the control greenhouse.

From time to time, free-hand sections were made of young leaves which developed under the above experimental conditions. The results will be given later in this paper.

On March 29, when the experiments ended, plants under the short-length days were photographed together with plants from all other conditions. All but two of the Abutilon plants under the five- and seven-hour length of day had a majority of uniformly dark green leaves as shown in pl. 9. A few old leaves in every case retained a somewhat faded mosaic pattern. These were already mottled before they were placed under the experimental conditions. In the case of the above exceptions, the plants were placed under the short-day treatment after the experiments were well under way, because they exhibited a very severe form of the chlorosis. While the intensity of the yellow color of the chlorotic areas became less in the younger leaves, these parts never approached the green condition as completely as did the others. In no instance did a plant become entirely free from all suggestion of the former variegated state. The faintest trace of a chlorotic condition could usually be observed in the younger foliage. After a time this became invisible so that the mature leaf was uniformly deep green. Minute isolated brown specks of dead tissue remained which did not occur in the green foliage of the control variety under the short days, so that in all probability we are correct in believing that these are due to the infectious chlorosis.

In the case of the varieties of *Evonymus japonica*, individual plants were equally liable to send out (1) a shoot entirely green, or (2) one entirely chlorotic. While the foliage in some plants of the var. "*aurea*" became more uniformly green under the

continuous illumination, there were plants in which the leaves became more deeply variegated. No generalizations could be drawn by attempting to attribute these characteristics to the influence of known environmental factors. In the short-day-length experiments the plants did not change their normal appearance.

Table III summarizes the plant material placed under each experimental condition.

SUMMARY OF THE RESULTS FROM DAY-LENGTH EXPERIMENTS

The chief observations from these studies under several degrees of illumination are as follows:

1. Under no conditions did the variegated varieties of *Evonymus* display the characteristics exhibited by *Abutilon striatum* var. *Thompsonii*.

2. While the young leaves of the control plants of *Abutilon striatum* var. *Thompsonii* became regularly infected, under continuous illumination they first became entirely green but those formed after the first two weeks became regularly and heavily infected.

3. New foliage appearing upon plants held under the five- and seven-hour-day lengths became entirely green in some cases and in other cases there was a strong tendency for the chlorotic areas to become fewer in number and reduced in size.

4. In no instance did the chlorotic areas of variegated leaves expand to embrace the entire leaf, that is, to cause the leaf to become uniformly yellow throughout.

THE EFFECT OF TOTAL DARKNESS

Four variegated plants each of *Abutilon Thompsonii*, *Evonymus japonica* varieties "*medio-picta*" and "*aurea*" were placed in the dark-room where the temperature and humidity were controlled as in the light experiments. Plants of *Abutilon* were the only ones to defoliate rapidly under these conditions. Two were taken into the light as soon as the stems were bared of leaves. The other two plants were kept in the dark for two weeks in which time they developed new top growth with etiolated leaves.

Each of the *Abutilon* plants taken in the light developed numerous small green leaves along the stem, and the greater

TABLE III

DAY-LENGTH EXPERIMENTS

Species and varieties	24 hrs. per day, continuous-light, + gas, temp. 78° F.	12 hrs. sunlight, 12 hrs. artif. light, gas, temp. 78° F.	19 hrs. dark, 5 hrs. light, temp. 78° F.	17 hrs. dark, 7 hrs. light, temp. 78° F.	12 hrs. dark, 12 hrs. light, temp. 78° F.	Control 78° F.
Abutilon striatum var. "*Thompsonii*"	6 plants	14 plants	4 plants	4 plants	4 plants	14 plants
Abutilon hybridum (green)	3 plants	2 plants	1 plant	1 plant	1 plant	2 plants
Evonymus japonica						
var. "*aureo-variegata*"	5 plants	10 plants	2 plants	2 plants	2 plants	13 plants
var. "*medio-picta*"	5 plants	10 plants	2 plants	2 plants	2 plants	13 plants
var. green	2 plants	5 plants	1 plant	1 plant	1 plant	5 plants
Total number of plants under each experiment	21	41	10	10	10	47

number of these later became severely chlorotic and remained dwarfed and sickly. The two plants which were kept in the dark-room for a longer time were eventually taken into the continuous-light room where it was noticed that the etiolated leaves became uniformly green and remained so. However, new leaves that were allowed to develop at the growing tip of the stem in the light became variegated and remained so, but the originally etiolated leaves which later developed chlorophyll never became visibly reinfected. From the experience of the author the effects which Baur attributes to the influence of complete darkness may have been due primarily to the loss of variegated foliage, a factor which will be considered in a later experiment.

HISTOLOGICAL STUDIES

HISTOLOGICAL STUDIES OF ABUTILON THOMPSONII SUBJECTED TO VARIOUS CONDITIONS OF LIGHT

It is well known, histologically, that variegations may be of two types, those in which the green and the chlorotic areas are arranged in sharply differentiated sectors and layers such as the so-called chimeras; or those in which the chlorotic and the green areas are not sharply defined, the affected cells exhibiting chloroplasts in all stages of dissolution from normal plastids to complete disintegration of the contents.

In the present discussion we are chiefly interested in the latter group to which *Abutilon Thompsonii* and other infectious chloroses belong. In the leaves of this plant there was a gradual transition from the normal green tissue to chlorotic, as free-hand sections of living leaf tissue showed. No particular hyperplasia or hypoplasia could be noticed in sections through chlorotic and normal areas. Although intensely variegated material was studied under ordinary magnifications of a high-power dry objective, no consistent differences in structure could be observed between the green and the chlorotic areas, thus confirming the earlier observations of Zimmerman ('92) and Baur ('08).

Method.—Cytological material was obtained from the plants from all previously described experiments, including the control plants, which can be collectively referred to as light treatments, except the experiments which were carried out in the spectral

houses. Care was taken that the sampling should be comparable in so far as the age and the chlorotic appearance of the leaf were concerned. As an additional precaution the length, width, and the rank of each leaf sampled was recorded for each plant, together with a simple description of its variegated appearance.

The fixing agents were made up as follows:

1. Formalin (40 per cent) . . . . . 5 cc.
   Alcohol (75 per cent by vol.) . . . . . 100 cc.
   Glacial acetic acid . . . . . 5 cc.
2. Corrosive sublimate, saturated in 50 per cent alcohol.

In the former fluid, the tissues may be stored indefinitely or until they can be conveniently dehydrated and imbedded in paraffin. The latter fixing agent was used while hot. The excess mercuric chloride was precipitated with iodine solution in the process of dehydration to 80 per cent alcohol.

Only a small proportion of the total material collected throughout the course of the light treatments has been sectioned and stained to date. The present data represent a portion of the material of *A. Thompsonii* which was collected between February 1 and 16, which included exactly one-fourth of the duration of the light treatments. The slides were stained with Haidenhain's iron-alum haematoxylin and counterstained with orange G. They were studied with a Leitz microscope equipped with a 1/10a fluorite or semi-apochromatic objective, with a numerical aperture = 1.30. All the drawings given in pl. 5 were made with the aid of a Spencer camera lucida and are of equivalent magnifications. It is intended that the drawings be considered semi-diagrammatic.

Observations.—Striking modifications were found in the leaf tissue of plants which were subjected to the light from the gantry crane in addition to daylight. Figure 2 of pl. 5 shows that the leaf at the beginning of the experiment was nearly normal except for the presence of unusually large intercellular spaces even in the palisade layer. Figure 1 of pl. 5 shows the condition in a leaf of an age comparable to that in pl. 5, fig. 2, after the experiment had been running for two weeks. The outstand-

ing modifications in the anatomical structure as a result of the continuous light are: increase in size of cells, increase in number and size of intercellular spaces, both of which contribute to the increase in thickness of the leaf. In addition, the cells have become literally packed with chloroplasts, so that it may be concluded that continuous light brings about an increase in number of chloroplasts as well as in thickness of leaves.

As opposed to the modifications in leaf anatomy which were brought about by continuous lighting in which daylight is supplemented by the gantry crane, are those resulting from subjecting the plants to the short or five-hour day. Figure 5 in pl. 5 shows the condition at the beginning of the experiment, and, as would be expected, is quite similar to fig. 2, pl. 5, both of them representing leaf structure after the experiment had been under way but a few days. Figures 6 and 7 represent the situation two weeks later and present modifications exactly the opposite to those found in leaves of plants which were kept under continuous illumination. The cells are a great deal smaller, and the intercellular spaces have become essentially negligible even in so short a time. The palisade layer is scarcely discernible. Chloroplasts appear to be smaller and fewer in number than in the cells at the beginning of the experiment. It was difficult in fixed material to distinguish between chlorotic and green areas.

In no case were cellular inclusions of the nature of the X-bodies of tobacco and other members of the Solanaceae infected with true mosaic disease observed in the Abutilon material which has been studied.

EXPERIMENTS ON THE INFECTIOUS PROPERTIES OF VARIEGATIONS

INOCULATION EXPERIMENTS

To date, one of the fundamental distinctions between the "infectious chlorosis" of *Abutilon* and the mosaic disease of tobacco lies in the fact that the former has been transmitted only by grafting whereas with the latter infection results readily from inoculation with the juices of the diseased plants. Consequently, all the possibilities for bringing about infection by inoculation should be tried, since the results may lead to a closer understanding of the principles underlying some of the facts

which are being discovered in connection with the virus diseases of plants. There are things to be said in favor of a new type of inoculation experiment in which the atmospheric environment of the virus and the oxidation processes of the expressed juices are made to simulate the conditions in the living cell.

Elmer ('25) has reported successful transmissions of sugar-cane virus to tobacco and of other mosaics which have proved difficult to cross-inoculate, by using an inoculum mixed with a 30 per cent solution of acetone. Vinson ('27) claims that the virus of tobacco can be concentrated by acetone and regained from solution by precipitation and inoculated without loss of infectiousness. The plant materials which he used were frozen before the juice was expressed.

Materials and method.—Leaves of *Evonymus japonica* vars. "*aurea*" and "*medio-picta*" were treated in the following way. The yellow areas were cut out and considered separately from the green areas. Both the green and the yellow portions were frozen for forty-eight hours before the juices were expressed. In order to obtain the juice the leaf materials were ground in separate mortars to which were added 2 cc. of a 30 per cent solution by volume of one of the following: acetone, ethyl alcohol, glycerine, and toluene. The resulting mixture was inoculated immediately. Samples of juice were also obtained from leaves which were not ground up with reagents. The plants to be inoculated were trimmed of all shoots and branches except two main stems, in order to stimulate new growth. The tip of one branch was cut off preliminary to an inoculation with a hypodermic needle into the pith of the stem. Inoculations were made on September 18, 1926.

Sixteen plants were grouped into four lots of four each. Half of the plants in each lot received expressed juice plus one of the above-mentioned reagents, while the other half received unadulterated juice. Two samples of juice expressed from yellow areas, that is, one with and one without the addition of an organic solvent, were inoculated into various parts of the stem and leaves by the following methods. On each plant some of the leaves were scratched with a needle and the inoculum applied from a pipette. Old leaves and very young leaves were treated alike.

Stabs with the point of a scalpel were made along the stem in three places, beside the axillary buds near the base of the stem, midway, and near the growing apex. Inoculations of the juice were made in two of these wounds, and a paste of leaf pulp (the residue after maceration) was placed in a third wound and bound with tape. A hypodermic syringe was used to inject the inoculum under pressure into the pith of the stem, the succulent parts of the stem, and the petioles of leaves. The entire experiment was repeated, using green areas instead of the chlorotic, thereby making a total of thirty-two plants in the experiments.

Results.—There were no positive indications of the transmission of the chlorosis in any of these plants during the subsequent year and a half. Experiments in which the juice from crushed leaves of *Abutilon striatum Thompsonii* have been inoculated into green plants of *Abutilon* have not resulted in the transmission of the variegation.

GRAFTING AND BUDDING EXPERIMENTS

Grafting and budding experiments were carried on in March, 1927, with the same varieties of *Evonymus* that were used in the inoculation experiments. In most cases the grafted scions of the variegated and the green plants died after a period of two weeks. From a total of fourteen grafts four lived.

In the case of two of the grafted plants of the variety "*aurea*" which survived, there was a definite transmission of the infectious chlorosis from a variegated scion to a green stock six weeks from the date that the graft was made. The chlorosis began to appear on one of the first leaves to mature, and at first it could be distinguished on close examination by a clearing of the larger veins. After two more weeks had elapsed, the chlorosis had traversed to all of the smaller vascular elements which lead to the interstices between the veins. By strong transmitted light it appeared as an indefinite mottling in the interstices. It was expressed uniformly throughout the entire leaf and was differentiated into very indistinct chlorotic and green areas, the outlines of which could scarcely be distinguished because of their minute size. Neither Bouché ('71), Baur ('08), nor Rischkow ('27b) have included in their papers photographs of the chlorosis

with which they were dealing, but from their descriptions it would seem that the chlorosis just described is identical with theirs. A photograph taken by reflected light of detached leaves exhibiting this peculiar type of infectious chlorosis, also a variegated leaf of the variety "*aurea*" from which the infectious chlorosis was obtained by grafting, is shown in pl. 11. Plate 11, fig. 2, also includes a photograph of a detached leaf from the non-infectious variety, "*medio-picta.*"

Of the four successful grafts which survived, two others, one of "*medio-picta*" on a green stock and one of green on a stock of the variety "*aurea,*" did not show any transmission of the chlorosis during the following eighteen months.

In addition to the successful transmission of the infectious chlorosis by grafting, it has been possible to transmit the chlorosis also by budding. Dormant buds of the variety "*aurea*" were inserted into the bark of ten normal green plants of *Evonymus*. A fairly high percentage of the buds lived for more than one month but eventually died. Two months after the death of the buds, on one of the plants the typical symptoms of this infectious chlorosis began to appear on the younger mature leaves.

Although the author has been unable thus far to graft variegated varieties of *Abutilon* with the green, there is no reason to believe that such transmissions of the chlorosis for the variety "*Thompsonii*" should prove impossible. Plate 7, figs. 2 and 3 are taken from figures of Lindemuth ('99a) and ('07) respectively, and show definitely that he was able to transmit the chlorosis of *Abutilon Thompsonii* to other members of the Malvaceae. Plate 8, fig. 1, is a photograph of infectious chlorosis on *A. megapotamicum,* which is included in order to show an infectious chlorosis on another species of *Abutilon.*

EFFECT OF THE REMOVAL OF VARIEGATED LEAVES

It has been reported by Baur that *Abutilon* infected with chlorosis can be made to lose all evidence of the disease by stripping off repeatedly all the leaves from the stem. Then by removing the leaves which show infection from each new crop for several successive crops, the leaves of the plant will eventually

develop and remain normal green without further evidence of previous infection. This is an experiment which he accomplished in the light.

It has been the writer's experience that this "curing" process can be accomplished but that it is accomplished gradually over quite a period of time. In the greenhouses at the Boyce Thompson Institute, Doctor L. O. Kunkel has had strongly variegated plants of *Abutilon Thompsonii* under his observation for three years. With one of them he followed the procedure which Baur used and removed the variegated leaves. The leaves on the other plant were allowed to remain on the stem. The photograph in pl. 11, fig. 1 was taken by the writer April, 1927, and shows the condition of the plant after three years. According to Baur, the "cured" plant on the right in the picture would be susceptible to subsequent infection if grafted with *Abutilon Thompsonii*, an experiment which has not as yet been carried out by Doctor Kunkel. The obvious experiment of grafting the "cured" stem back upon a green susceptible stock of *Abutilon* has not been tried, although it is acknowledged that such an experiment is necessary before it can be established conclusively whether or not a variegated individual can be "cured" and retain and transmit the infectious agency.

Biochemical Studies

VARIEGATED LEAF PIGMENTS

The author held the view that quantitative determinations of variegated leaves might present data which would indicate the presence of chemical disturbances affecting the normal molecular ratios of chlorophyll a/chlorophyll b and carotin/xanthophyll. Thus, certain indications might be gained as to the probable sequences in the development of chlorosis.

Quantitative determinations of chlorophyll and carotinoid pigments have been made on the leaves of *Abutilon Thompsonii* and a green variety of *Abutilon*. The chlorophyll fractions were determined as phytochlorin-E and phytorhodin-G which were made up to volume with appropriate concentrations of hydrochloric acid and compared colorimetrically with Guthrie's standards (see Guthrie, '28). The data are withheld, however, until the results from further analyses will justify conclusions.

ACIDITY DETERMINATIONS

ELECTROMETRIC DETERMINATIONS OF HYDROGEN-ION CONCENTRATION

Apparatus.—By including the recently developed quinhydrone electrode system with the calomel cell which Hildebrand ('13) used with the normal hydrogen electrode, a remarkably inexpensive instrument, easy to operate, can be made for the determination of hydrogen ions. Such an apparatus was built by the author following the suggestions made by Dr. William Youden,[1] at the Boyce Thompson Institute.

Since in these experiments it was necessary to use a fraction of one cc. of the leaf juices for a single reading, and since for an ordinary platinum electrode there is needed at least 2 cc. of a solution, the method was modified to determine the acidity in a single drop of the sample. To do this a thin strip of copper foil about 2 cm. wide was used in the circuit. This served to support the platinum electrode which consisted of a flat plate of platinum foil carefully depressed on one surface to hold about one or two drops of the sample liquid. Contact between the platinum and the copper strip was assured by depressing the surface of the latter to correspond exactly with that of the platinum both as to shape of the indentation and as to its depth. Contact between the calomel electrode and the sample drop was made in the usual way by means of an agar salt bridge of saturated potassium chloride solution. However, care was taken to avoid a siphon action in all cases where the solution of potassium salt was used, by plugging the open end which extended into the calomel cell with cotton and by drawing the opposite end to a capillary point.

Method.—The hydrogen-ion concentrations of leaf juices expressed from the green and lighter pigmented areas of variegated leaves have been determined electrometrically by the quinhydrone method for all varieties of *Evonymus japonica*, representative leaves of which are shown in pl. 8, fig. 2. In all cases the initial acidity of the juice was determined quickly and recorded. In

[1] Dr. Youden perfected the "Youden Hydrogen Ion Outfit," which is being sold by the W. M. Welch Scientific Company of Chicago. For a complete description of the apparatus, including directions for the use of materials, the electrical instruments, and the mechanical features incident to the electrical wiring, the reader is referred to these authorities.

the case of certain samples the juice was permitted to stand in air and subsequent determinations were made from it at stated intervals of time varying from a few to several minutes.

The procedure was as follows: Leaves were taken from a particular variety of *Evonymus*, and in determinations where variegated leaves were used the dark and light areas were carefully cut out. These portions of the leaves were ground separately in individual mortars. The determinations were carried out at once upon the undiluted juices which were expressed by the grinding operation. Quinhydrone crystals, in a quantity sufficient to be easily carried on the pointed tip of a scalpel blade, were added to each sample before the determination was made. This mixture was quickly brought to a condition of equilibrium by stirring with the tip of a very fine glass rod. The capillary point of the salt bridge was placed in the drop of sample and a reading was taken immediately.

The difference in potential between the saturated calomel electrode standard and the quinhydrone electrode at the equilibrium of the system was observed as voltage on a Weston millivoltmeter. The pH of the sample was read directly from a table constructed from a formula relating the voltage of a calomel-quinhydrone system to pH, at a temperature of 25° C. When the room temperature ranged above this value the readings were corrected accordingly. The formula for the conversion of the quinhydrone electrode E.M.F. to pH, when a saturated calomel reference electrode = 0.2464 volts at 25° C. was used, is as follows:

$$\text{Formula:}\quad \text{pH} = \frac{.699 - \text{E} - .246}{.0591}$$

When solutions are more acid than pH = 7.66, the calomel is negative and the quinhydrone electrode is positive. Using solutions more alkaline than pH = 7.66, the calomel is positive and E has a negative value in the formula. The lead wires, *g* and *h* in pl. 10, connecting the voltmeter, *m*, and the galvanometer, *n*, with the calomel cell and the quinhydrone electrode respectively, depend for their respective positions upon the degree of acidity of the solution in question. When solutions are more acid than pH = 7.66, the calomel is connected with the circuit by

TABLE IV

COMPARISON OF INITIAL ACIDITY IN CHLOROTIC AND GREEN TISSUES

Variety used	No. of plants in sample	Green		Chlorotic	
		$(H+)^{10-6}$	pH	$(H+)^{10-6}$	pH
"medio-picta"	7	.363	6.44	.85	6.07
	7	.257	6.59	.85	6.07
	7	.295	6.53	—	—
	7	.388	6.41	.955	6.02
	1	.257	6.59	.456	6.34
	1**	.218	6.66	.575	6.24
	1**	.308	6.51	.757	6.12
	1*	.456	6.34	.708	6.15
	1	.257	6.59	.240	6.62
	19	.295	6.53	.575	6.24
"aurea"	5	.426	6.37	.363	6.44
	5	.388	6.41	.436	6.36
	1*	.234	6.63	.104	6.98
	1*	.456	6.34	.308	6.51
	1**	.218	6.66	.263	6.58
	1**	—	—	.288	6.54
	1*	.562	6.25	.794	6.10
	1*	.562	6.25	.562	6.25
	1**	.380	6.42	1.26	5.90
	1**	.436	6.36	1.35	5.87
	10	.380	6.42	.209	6.68
	4	.295	6.53	.380	6.42
	5	.295	6.53	.257	6.59
	1	.338	6.47	—	—
	5	.456	6.34	.436	6.36
"argenteo"	4	.812	6.09	1.41	5.85
	4	.536	6.27	1.32	5.88
	1	.812	6.09	.955	6.02
	4	.562	6.25	—	—
	1	.812	6.09	1.26	5.90
	1	.812	6.09	1.32	5.88
	1	1.00	6.00	2.22	5.65
	1	.562	6.25	1.78	5.75
green	1	.209	6.68	—	—
	1	.245	6.61	—	—
	1*	.186	6.73	—	—
	1**	.675	6.17	—	—
	1*	.257	6.59	—	—
	1*	.257	6.59	—	—
	1*	.288	6.54	—	—
	1*	.308	6.51	—	—
	1	.812	6.09	—	—
	1*	.234	6.63	—	—
	1*	.209	6.68	—	—
	31	.675	6.17	—	—
	31	.725	6.14	—	—

* = young and very young leaves; ** = old and very old leaves.

means of lead wire, *g*, and the quinhydrone-platinum electrode is

connected by means of *h*. They are interchanged for solutions more alkaline than pH = 7.66.

Tests were made repeatedly throughout the course of the experiments, using buffer solutions of known pH to ascertain the degree of accuracy of the single-drop method, as opposed to electrometric methods where larger samples of the buffers were used. The instrument was found correct within 0.03 pH.

Full directions for the preparation of calomel electrodes are given by Findlay ('20, pp. 228–230), and by Clark ('20, pp. 133–134). Many suggestions are made by these authorities which are of assistance in measuring acidity with the quinhydrone electrode system. It should be borne in mind that, because of its simplicity and the convenience with which it can be used in the field, a M/20 potassium acid-phthalate solution makes probably the most satisfactory reference electrode that the biologist can use.

Results.—The data from the hydrogen-ion determinations are given in tables IV, V, VI, and VII. The results which are included in these tables are a fair representation of the results from nearly 400 determinations, many of which are not given in this paper for lack of space.

TABLE V

COMPARISON OF YOUNG AND OLD LEAVES AS TO INITIAL ACIDITY

Variety used	No. of plants in sample	Young leaves				Old leaves			
		Green		Chlorotic		Green		Chlorotic	
		$(H+)^{10-6}$	pH	$(H+)^{10-6}$	pH	$(H+)^{10-6}$	pH	$(H+)^{10-6}$	pH
"medio-picta"	1	.257	6.59	.456	6.34	.218	6.66	.575	6.24
	1	.456	6.34	.562	6.25	.308	6.51	—	—
"aurea"	1	.234	6.63	.166	6.78	.245	6.61	.263	6.58
	1	.456	6.34	.308	6.51	—	—	.288	6.54
	1	.562	6.25	.794	6.10	.380	6.42	1.26	5.90
	1	—	—	—	—	.436	6.36	1.35	5.87
"argenteo"	1	.812	6.09	1.32	5.88	1.00	6.00	1.78	5.75
	1	—	—	—	—	.562	5.25	1.78	5.75
	1	—	—	—	—	—	—	2.22	5.65
green	1	.186	6.73	—	—	.675	6.17	—	—
	1	.256	6.59	—	—	—	—	—	—
	1	.256	6.59	—	—	—	—	—	—
	1	.288	6.54	—	—	.308	6.51	—	—
	1	.234	6.63	—	—	.209	6.68	—	—

CHLOROTIC AND GREEN AREAS

A comparison of the initial acidities for the green and the chlorotic areas of all the variegated varieties of *Evonymus* included in the present paper indicates that the chlorotic areas are higher in initial acidity than the green for all varieties except "*aurea.*" Arranged in the order of descending values, they are "*argenteo,*" "*medio-picta,*" and "*aurea.*" The data suggest that in the white variegated variety, "*argenteo,*" the chlorotic areas have a consistently higher initial acidity than the chlorotic areas of any other variety.

YOUNG AND OLD LEAVES

While the chlorotic areas were found to have a higher initial acidity in old leaves than in young leaves, the green areas were more acid. Old leaves of the normal green variety were higher in (H^+) concentration than the young leaves.

EFFECT OF FREEZING

Freezing the leaves just prior to expressing the juice increased the acidity of the juice of both the green and the chlorotic areas. For data in addition to that given in table VI reference should

TABLE VI

EFFECT OF FREEZING ON INITIAL ACIDITY

Variety used	No. of plants in sample	Frozen				Non-frozen			
		Green		Chlorotic		Green		Chlorotic	
		$(H+)^{10-6}$	pH	$(H+)^{10-6}$	pH	$(H+)^{10-6}$	pH	$(H+)^{10-6}$	pH
"*medio-picta*"	1	.275	6.56	.812	6.09	.257	6.59	.575	6.24
	1	.308	6.51	.812	6.09	.256	6.59	.600	6.22
green	1	.346	6.46	—	—	.209	6.68	—	—

be made to text-figs. 2 and 3, in which the initial acidity determinations in the titration experiments on juices from frozen and fresh tissues exhibited the same phenomenon.

EFFECT OF EXPOSURE TO AIR

As mentioned in the discussion of the method, several determinations were made at stated intervals of time upon samples of

juice which were being exposed to air. It was observed that for all samples of juice there was a decided increase in the acidity which was roughly proportional to time, as is indicated by the

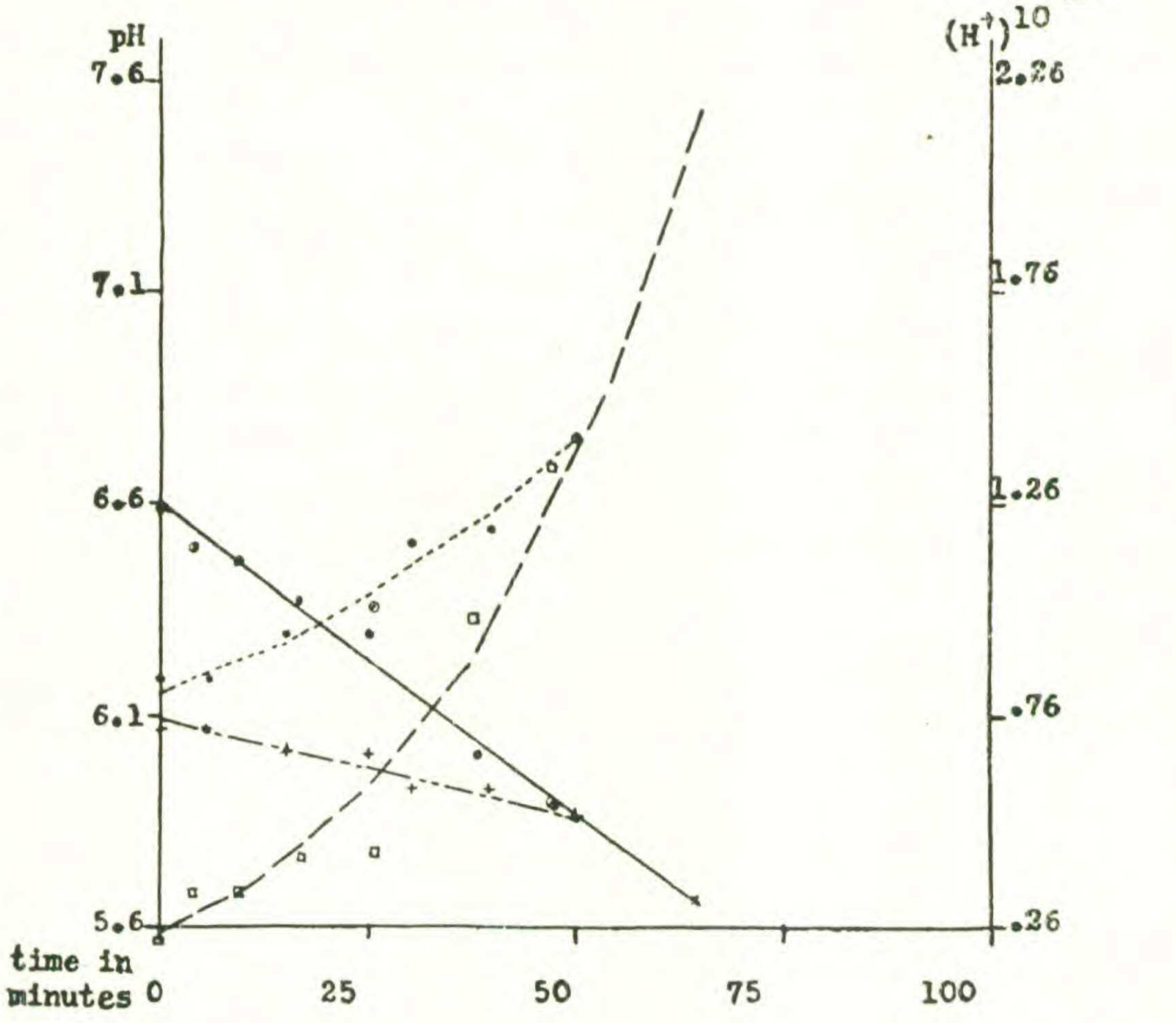

Fig. 1 Progressive changes in pH and (H^+) with time of exposure of juice to air.

data furnished in table VII and text-fig. 1. It will be noticed at once from the points on the graph that the pH of the juice from green and chlorotic samples decreased steadily throughout

the course of the experiment; that is to say that the acidity of the juice increased steadily. The points on the graph, where pH is plotted against time for both green and chlorotic leaf areas

TABLE VII

EFFECT OF EXPOSURE TO AIR ON THE ACIDITY OF EXPRESSED JUICE

Variety used	No. of plants in sample	Green			Chlorotic		
		Time min.	$(H+)^{10-6}$	pH	Time min.	$(H+)^{10-6}$	pH
"*medio-picta*"*	7	0	.257	6.59	0	.85	6.07
		4	.324	6.49	6	.85	6.07
		9	.338	6.47	15	.955	6.02
		17	.426	6.37	25	.955	6.02
		26	.436	6.36	30	1.17	5.93
		38	1.00	6.00	39	1.20	5.92
		47	1.35	5.87	50	1.41	5.85
"*aurea*"	5	0	.426	6.37	0	.218	6.66
		4	.675	6.17	6	.257	6.59
		9	.708	6.15	12	.295	6.53
		15	.795	6.10	21	.308	6.51
		23	.955	6.02	26	.436	6.38?
		28	1.00	6.00	32	.537	6.27
		36	1.07	5.97	36	.575	6.24
		46	1.20	5.92	47	.812	6.09
		96	1.74	5.76	62	.955	6.02
"*argenteo*"	4	0	.537	6.27	0	1.32	5.88
		3	1.26	5.90	6	1.32	5.83
		5	1.26	5.90	10	1.55	5.81
		8	1.48	5.83	15	1.58	5.80
		14	1.62	5.79	21	2.18	5.66?
		18	2.09	5.68	24	1.74	5.76
		24	2.63	5.58	29	1.95	5.71
		28	2.95	5.53	37	1.99	5.70
		34	2.88	5.54	55	2.18	5.66
		37	3.08	5.51	65	2.45	5.61
		41	3.08	5.51	—	—	—
green	1	0	.209	6.68			
		2	.199	6.70			
		5	.209	6.68			
		9	.257	6.59			
		15	.288	6.54			
		19	.380	6.42			
		23	.388	6.41			
		27	.513	6.29			
		41	1.00	6.00			

* Data show agreement with theoretical linear curves to within an average deviation of 0.03. See text-fig. 1.

of the variegated variety "*medio-picta,*" show a close agreement with a linear function when they are fitted in the equations

$$(1) \quad m = \frac{n\Sigma(xy) - \Sigma x y}{n\Sigma(x^2) - (\Sigma(x))^2}$$

$$(2) \quad b = \frac{\Sigma y - m\Sigma(x)}{n}$$

and when these values are further substituted in the equation

$$(3) \quad y = mx + b$$

y = actual pH
x = time interval
m = factor for equation (1)
b = value for equation (2)

The deviation of each point from the straight-line curve is expressed as the difference between the actual pH values determined in the experiment and the theoretical values for pH as calculated from the above equations. These numerical values are shown in table VIII. In text-fig. 1, the average deviation of all points from the theoretical curve is less than 0.03 pH, which is in agreement with the limits of the experimental accuracy for this type of potentiometer and with the method used in making the determinations.

ELECTROMETRIC DETERMINATIONS OF TOTAL ACIDITIES

Apparatus.—In addition to the electrical instruments used in the determinations of hydrogen-ion concentrations by the quinhydrone electrode method, the apparatus was modified to include the following additional equipment, as shown in pl. 10.

A platinum electrode was made by sealing a triangular piece of platinum foil, measuring from 1.5 to 2.0 cm. from base to apex, into the molten end of a glass tube, *a*, having a bore of approximately 3 mm. The tube was partially filled with mercury to insure a suitable contact between the platinum foil and an insulated copper lead wire about number 26 D.S.C. The necessity for rigging a separate stirring device was alleviated by rotating the glass tube, *a*, in the vial, *b*, which also contained the quinhydrone-platinum electrode system, one end of the salt bridge, *c*, the tip of the burette, *d*, and the plant juice which was to be titrated. The saturated calomel cell, *e*, was indirectly

TABLE VIII

ACTUAL AND THEORETICAL VALUES FOR pH AND (H^+) FOR DATA PLOTTED IN TEXT FIG. 1

Tissue	pH				$(H^+)^{10-6}$			
	Time min.	Theoretical value	Actual value	Difference	Time min.	Theoretical value	Actual value	Difference
Green	0	6.60	6.59	.01	0	.251	.257	.006
	4	6.54	6.49	.05	4	.288	.324	.036
	9	6.47	6.47	.00	9	.339	.339	.000
	17	6.35	6.37	.02	17	.447	.426	.021
	26	6.22	6.36	.14	26	.603	.436	.167
	38	6.04	6.00	.04	38	.912	1.00	.088
	47	5.89	5.87	.02	47	1.29	1.35	.06
	54	5.81	—	—	54	1.55	—	—
	59	5.73	—	—	59	1.86	—	—
	64	5.66	—	—	64	2.19	—	—
Chlorotic	0	6.09	6.07	.02	0	.812	.85	.038
	6	6.06	6.07	.01	6	.87	.85	.02
	15	6.03	6.02	.01	15	.932	.955	.023
	25	5.98	6.02	.04	25	1.04	.955	.085
	30	5.95	5.93	.02	30	1.12	1.17	.05
	39	5.91	5.92	.01	39	1.23	1.20	.03
	50	5.80	5.85	.01	50	1.41	1.41	.000

connected with the vial, *b*, by means of the salt bridge, one end of which dipped into a saturated KCl solution in vial, *f*. This solution was also in contact with the tip of the side-arm of the calomel cell. When in use, the salt bridge must be filled with a saturated solution of KCl, the end in vial, *f*, may be plugged with cotton, and the opposite end in vial, *b*, may be drawn to a capillary fineness to prevent a siphon action between the vials.

Lead wires, *g* and *h*, connecting the voltmeter, *m*, and galvanometer, *n*, with the calomel cell and the quinhydrone electrode respectively, must be interchanged during the course of the titration according to the degree of acidity of the solution under examination. For the procedure with solutions more alkaline than pH 7.66, directions have been given in the discussion of electrometric determinations of hydrogen-ion concentrations. Reference has also been made to the sources of information concerning the use of materials and electrical instruments, as well as to the mechanical features incident to the electrical wiring.

Power for turning the stirring apparatus was furnished by means of a small electric motor, *i*, to which the pulley and cords were arranged as shown in pl. 10. Using the glass tube of the platinum electrode as a stirring rod and the exposed blade of the platinum foil as a paddle necessitated an arrangement providing for the rotation of the shaft, which must be held in place and rigidly supported in order to insure a true circular motion. The shaft was made to fit snugly into the bore of a short piece of glass tubing, *k*, which shall be referred to as a sleeve. The sleeve, although carefully selected to avoid undue friction and too much play, was lubricated with vaseline, and when in operation served as a well-lubricated bearing. The sleeve was fitted tightly into a one-hole rubber stopper which was clamped securely to an iron stand. In a similar way, the shaft was fitted into a one-hole rubber stopper which plugged a hole centrally located in a pulley rotating in a plane perpendicular to the shaft. A metal washer, *l*, was inserted between the rubber stoppers to increase efficiency.

For the titration of 1- to 2-cc. quantities of plant juice, it was necessary to use a burette of very small dimensions. A satis-

factory burette[1] can be made from a straight pipette having a capacity of 2 cc. and graduated in hundredths cc.

Materials and methods.—The plant materials used were the leaves of *Evonymus japonica*, variety green and variegated varieties "*aurea*" and "*medio-picta.*" The juice was expressed by means of a hydraulic press at a pressure approximately equal to 10–15 pounds per square inch. The juice was always filtered quickly through cheese-cloth and titrated at once or allowed to stand, depending upon the conditions of the experiment. In some instances the leaves were frozen at a temperature of −19° C. in a cold room before pressure was applied. In other experiments the leaves were permitted to stand for an equal period in a room where the temperature was cool, but never fell below 5° C. The experiments were carried on during the month of August in Yonkers, N. Y. The leaves were stripped from plants which had been growing in an outside garden for three months.

Twentieth normal and fiftieth normal NaOH and HCl solutions were made up from reagents of C.P. quality and stored in Jena flasks with ground-glass stoppers, for the duration of the experiments. Titration curves of N/20 and N/50 NaOH with HCl are included with the data from the titration of NaOH against the plant juice extracts.

EFFECT OF FREEZING ON BUFFER ACTION

Method.—Variegated leaves of *Evonymus japonica* var. "*aurea*" were collected at 6:00 P.M. and were then divided into two lots. The leaves of one lot were kept entire. The second lot was subdivided, the yellow areas being cut out from the green and considered separately. Each of the three lots of tissue (entire leaves, yellow areas, and green areas) were divided equally, one-half being left to freeze over night, the other half kept above freezing for the same interval of time. During the course of the following day, juice was expressed from each fraction and titrated immediately with N/50 NaOH. Duplicate titrations were made on each sample and the resulting curves showed remarkably

[1] The burettes, *d*, which are shown in pl. 10, were made up for the author by Eimer & Amend Company in New York.

close agreement. The results from the titrations are shown in text-fig. 2.

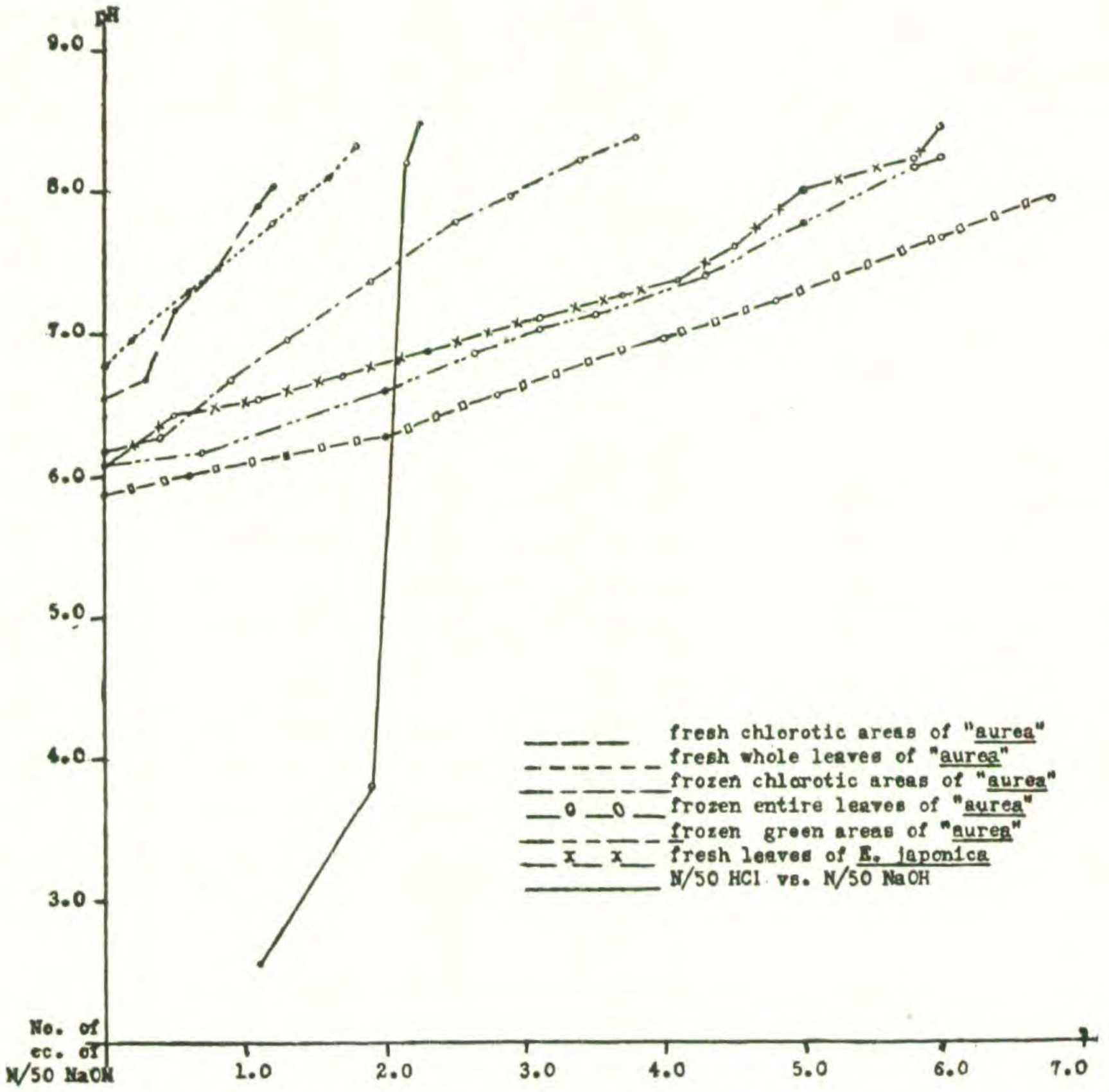

Fig. 2. Titrations of *E. japonica* var. "*aurea*" and of *E. japonica* with N/50 NaOH.

Similar experiments were conducted on variegated leaves of *E. japonica* "*medio-picta.*" The titrations were made with N/20 NaOH and in some cases with N/50 NaOH. Representative curves from the titrations with N/20 NaOH are shown in text-fig. 3.

Results.—The graphs in text-figs. 2 and 3 show that variegated varieties of *Evonymus japonica* group themselves into two distinct categories, namely, those exhibiting pronounced and prolonged buffer action, and those exhibiting less pronounced and prolonged buffer action. To the first category belong all titration curves

of juices expressed from frozen leaf tissue. To the second belong all titration curves of juices expressed from fresh or non-

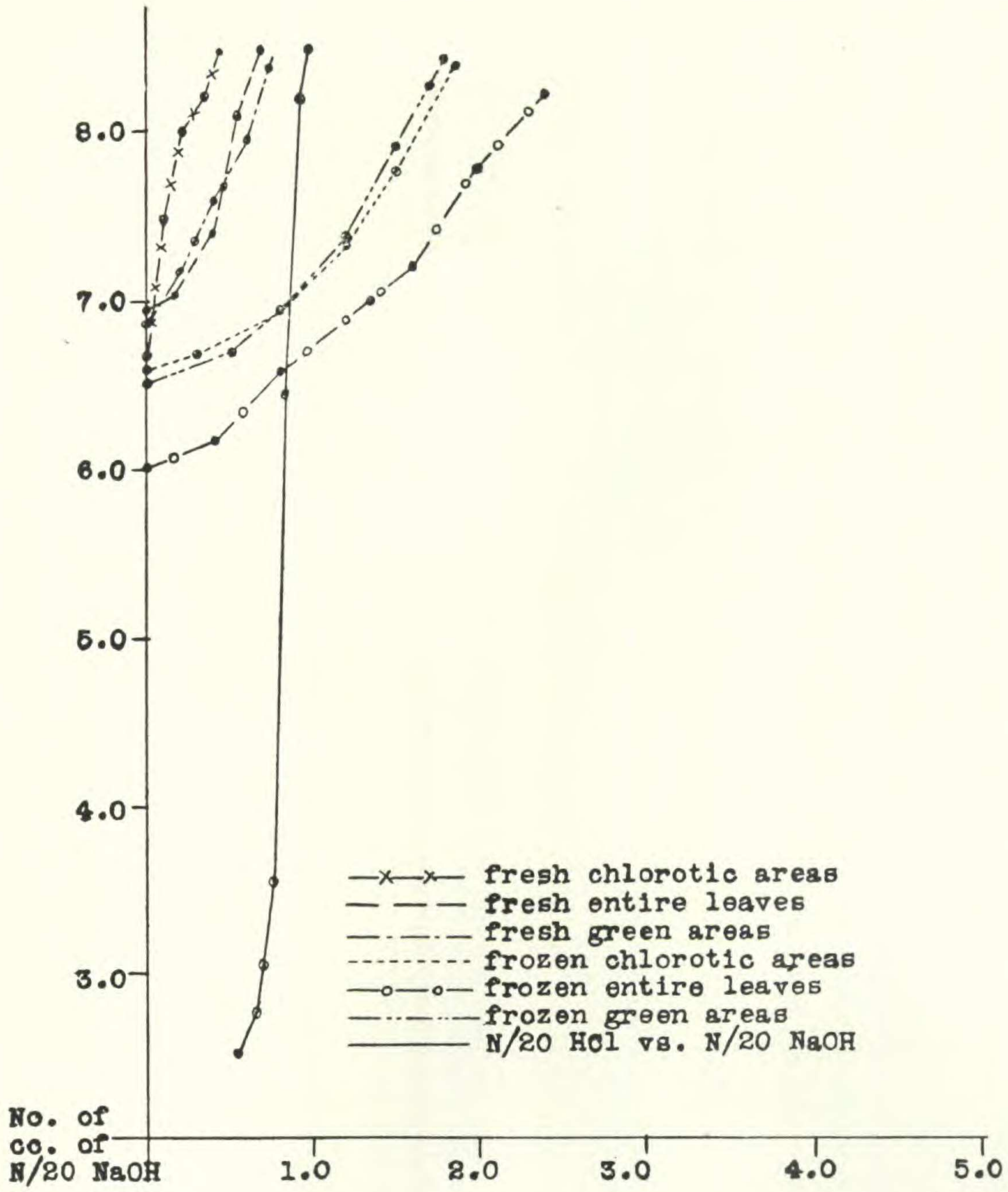

Fig. 3. Titrations of *E. japonica* var. "*medio-picta*" with N/20 NaOH.

frozen leaf tissue. Titration curves of the juices expressed from fresh leaves of the green variety fall into the first category along with curves for the titration of juices of frozen variegated leaves.

Many more titrations were made than were plotted here, without the occurrence of a single exception to the case in point.

EFFECT OF EXPOSURE OF EXPRESSED JUICE TO AIR

Method.—In this experiment part of the juice expressed from variegated and green leaves of *E. japonica* was titrated immedi-

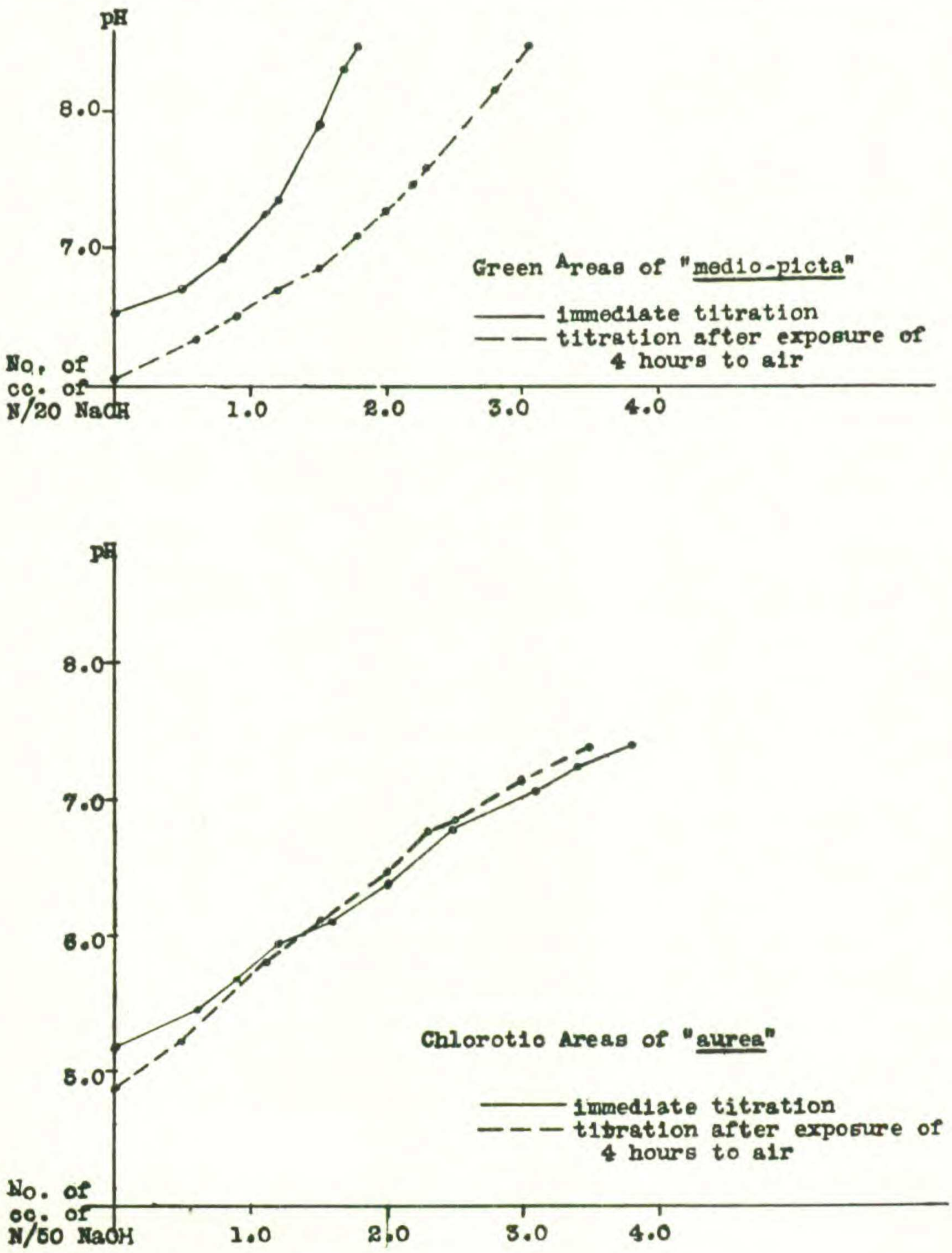

Fig. 4. Effect of exposure of expressed juice to air.

ately, while the other part was set aside and exposed to air for intervals of time varying from four to twelve hours, and then titrated.

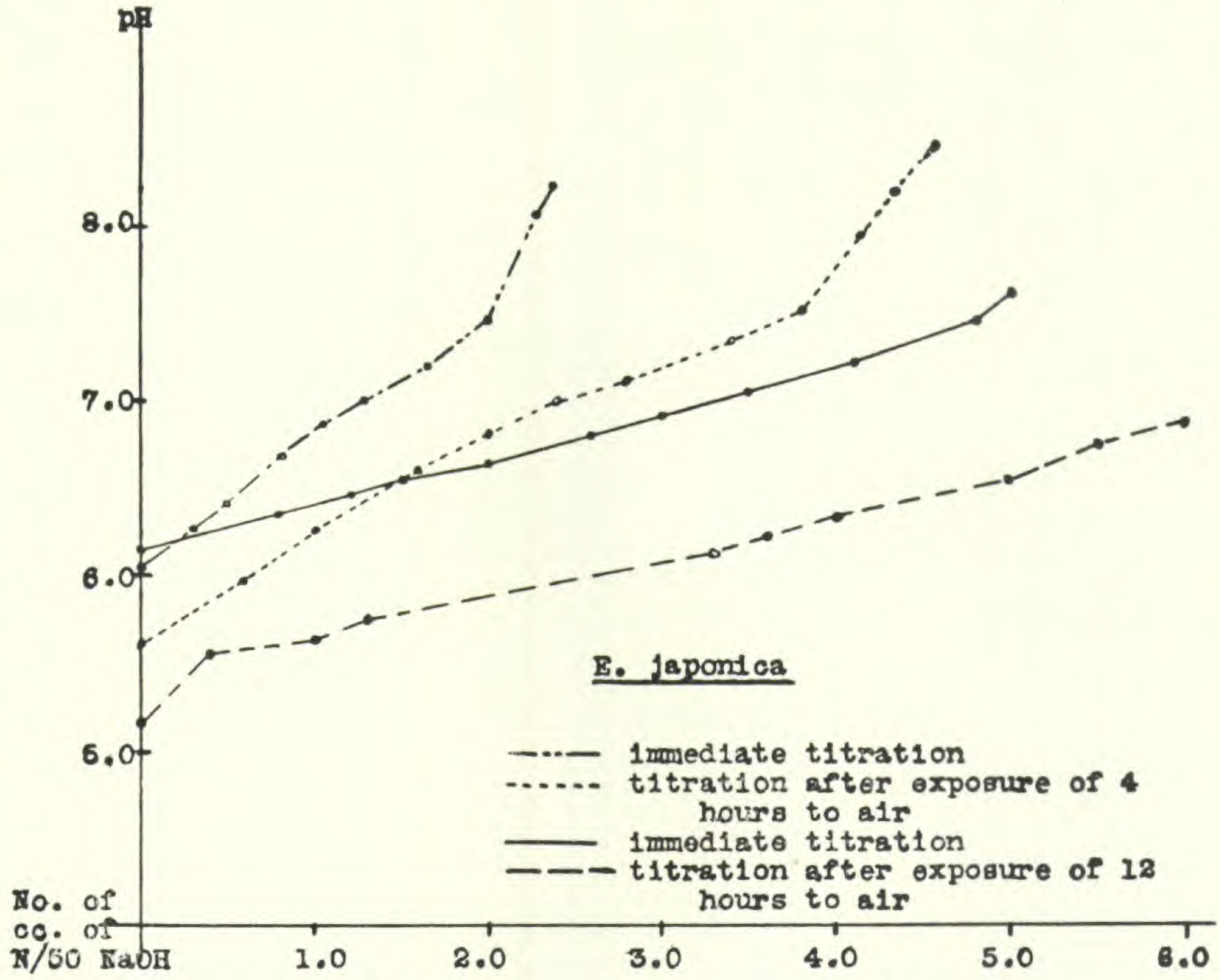

Fig. 5. Effect of exposure of expressed juice to air.

Results.—The initial acidity determinations as shown in text-figs. 4 and 5 all point to one conclusion, namely, that the juices from entire green leaves of the green variety and from the chlorotic and green portions of the leaves of the variegated varieties became more acid on standing in an atmosphere of air. It is clearly indicated on the graphs that a similar relationship also holds for the entire titration except in juices from chlorotic areas. That is to say, the curves, though not identical, run parallel; a fact which indicates that during the course of the titrations of the samples exposed to air for four to twelve hours there resulted a consistently lower pH when equal quantities of N/50 NaOH were added to samples from green areas. The author has no

data to show whether or not the same holds true for the entire leaves of variegated individuals.

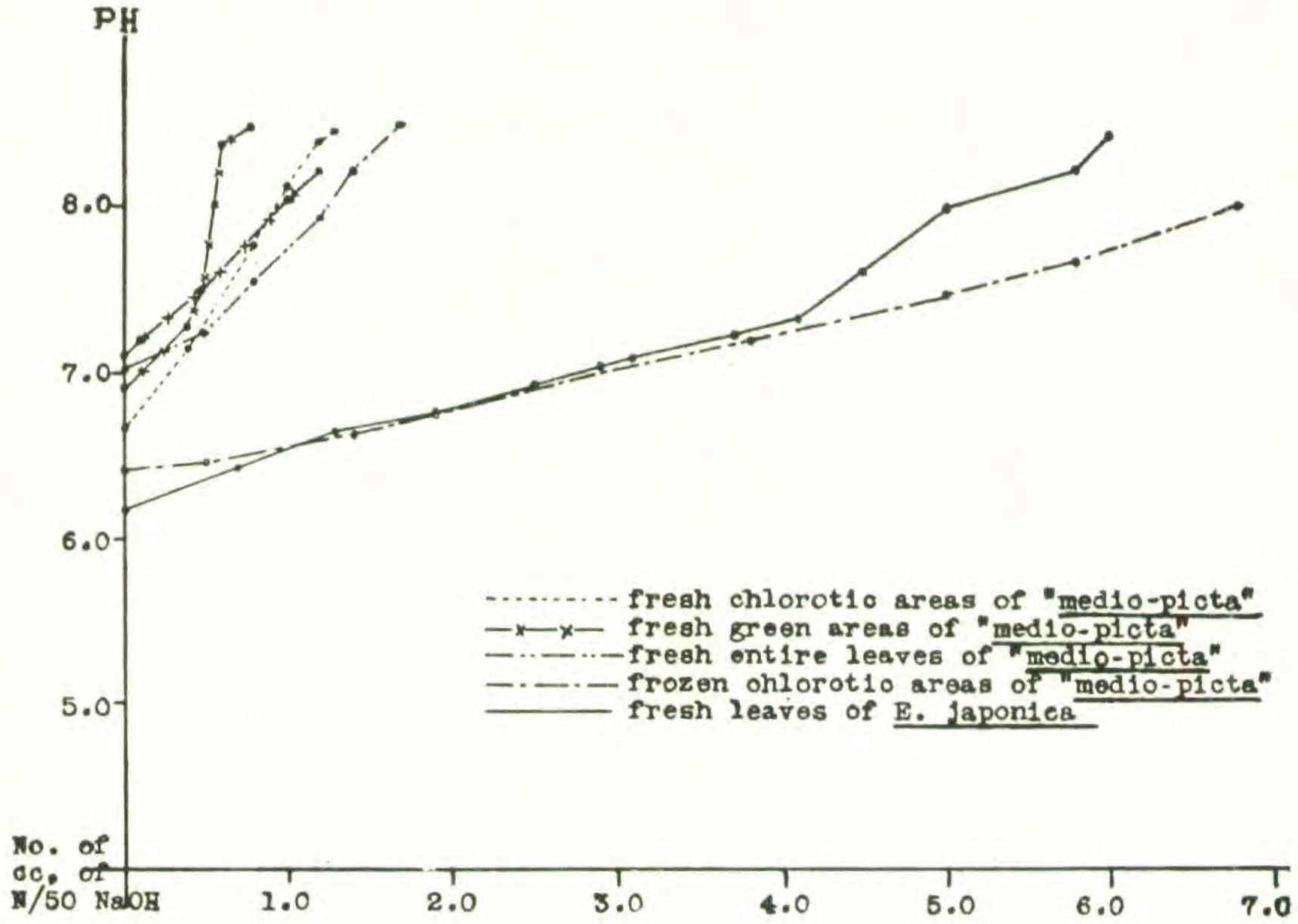

Fig. 6. Titrations of *E. japonica* var. "*medio-picta*" and of *E. japonica*.

DISCUSSION

The data obtained from the foregoing experiments have a direct bearing upon the conclusions of certain earlier authors whose results were presented in the "Discussion of Literature" in this paper. From the author's own work and that of Baur, it is clear that the condition of mottling in infectious chloroses can be produced under light in both halves of the spectrum. The observations of Baur ('06a) with reference to the loss of the chlorotic appearance as a result of exposure of plants to light of various wave-lengths could not be substantiated in the case of *Evonymus japonica* var. "*aurea.*" Although the writer observed that leaves of *Abutilon Thompsonii* became quite uniformly green during the five-months exposure to sunlight deficient in blue rays under one set of conditions, and green rays under another, he is inclined to favor the view that these effects may be attributed partially or entirely to shade which was produced

under Corning's glasses Noviol "C" and G 34, as this factor could not be successfully eliminated during the winter months.

In considering further the influence which light may have on the expression of the infectious variegation, it might be argued that increased illumination and possibly continuous exposure to light of high intensity might favor the spread of the chlorotic condition of infectious chlorosis to all parts of the leaf. In so far as it can be ascertained from experiments of high-light intensity and from the continuous exposure of the infected plants to this intensity, there is no physiological factor in the infected leaves of *Abutilon Thompsonii* and *Evonymus japonica* var. "*aurea*" which can be influenced by light to cause them to become entirely and uniformly yellow through the enlargement of the chlorotic areas.

Plants of *A. Thompsonii* receiving light for five- and seven-hour day lengths, as compared with those grown under identical conditions except for exposure to longer periods of day length, developed new leaves, of which each successive crop became more uniformly green until new leaves matured which were entirely free from the chlorotic condition. Comparable results were obtained by Baur when plants of *A. Thompsonii* were partially shaded from sunlight. Where Baur's results were obtained from few plants held under experimental conditions which were unsatisfactorily controlled, the results from the present study were obtained by experimenting with a large number of plants held under carefully controlled conditions. It is interesting to note that shade and short-day length have similar effects on the infectious variegations.

Baur emphasized the curing effects which exposure to darkness had upon plants infected with chloroses. Results given in the present paper show that such treatment, for short periods of time at least, was not sufficient to destroy the infectious property of the virus, as was evidenced by the fact that after a plant was returned to the light the new leaves which formed promptly became infected. However, when the plants were kept in the dark after defoliation of the mature leaves, new and etiolated leaves were formed which developed chlorophyll when the plants were brought into the light, and these remained on the stem in the vicinity of subsequently formed variegated leaves

without becoming infected. The fact that leaves which are formed in the dark will remain uninfected although they mature in the light may possibly be explained by some such hypothesis as Baur's theory of immunity. He pictured a type of immunity which would explain the fact that if leaves were prevented from becoming infected until they had attained a certain degree of differentiation and development, they would thereafter remain immune to infection. A plant of *A. Thompsonii* kept in the light can be restored permanently to a condition where it is uniformly green by removing the variegated leaves for several successive crops. These facts have led the author to the conclusion that the curing effects observed by Baur which he attributed directly to the absence of light may be due principally to defoliation.

The greening processes of variegated leaves may possibly be related to the absence of normal metabolic and synthetic activities of the plants under investigation. Whether the infectious agency can be inactivated or made to lose its power of reproduction by means of certain light treatments, or, on the other hand, the leaf tissues rendered effectively resistant to the invasion of the virus by such treatments, can be subjected to experimental proof. In the light of such a test, it would become a point of theoretical interest to follow the changes in the chemical constituents related to the metabolism of the leaf and to the photosynthate for certain stages of the greening processes. This work will be carried out in the future as time permits.

When samples of juice expressed from green and chlorotic portions of leaves of varieties of *Evonymus japonica* were exposed to air, and the hydrogen-ion concentrations were determined upon these at intervals of time varying from a few minutes to several hours, it was found that an increase of acidity of the juice was proportional to time. The author has been unable to attach any theoretical significance to the linear nature of the pH curves from the present data, or to account satisfactorily for the rapid fall of pH with time. The failure to transmit infectious chlorosis by other means than by grafting or budding makes it important to consider any changes that may be detected in the juices from the time that the macerated leaf tissue is exposed

to the air to the moment that inoculation is made into the susceptible green plant. Therefore, such changes in the hydrogen-ion concentration should be prevented, as far as possible, during inoculation experiments in cases where the inoculum ordinarily fails to transmit the infectious virus. Acidity experiments performed by Dr. B. M. Duggar, but not reported, showed a similar phenomenon in chlorotic and green areas of plants infected with true mosaic disease.

From results of electrometric titration experiments on juices of frozen and non-frozen leaves of varieties of *Evonymus japonica*, it can be concluded that freezing has increased buffer action in the juices expressed from these varieties. The data suggest the possibility that cells may be ruptured by the freezing process, with the result that the cell contents may be expressed more completely from the pulp under hydraulic pressure. Titration curves of data from experiments in which the titrations were made after the juices were allowed to stand in an atmosphere of air show a consistently lower pH when equal quantities of N/50 NaOH were added to samples from green areas. It is considered likely that a process of organic oxidation may be concerned here. Carbohydrates may be oxidized to form organic acids, a result which would bring about an increase in acidity which would be consistent with the data from similarly performed experiments with expressed juices where the free and the titratable acidities have been determined simultaneously.

There are certain contrasts in buffer action between variegated and green leaves of *Evonymus japonica* which have been clearly brought out by the foregoing experiments. From the data which have been presented here, it can be assumed that the tissues in yellow and green portions of variegated leaves exhibit distinctiveness in regard to the nature of their physiological processes. Additional evidence pointing to the same conclusion is not lacking from data of other titration experiments. This evidence will be briefly summarized in the following paragraphs.

Juices from the fresh or non-frozen variegated leaves of varieties *E. japonica "medio-picta"* and *"aurea"* show relatively little buffer action as compared with the juice from leaves of the green variety (see text-figs. 3 and 6).

The juices from frozen variegated leaves show a significant increase in buffer action over and above that for juice from fresh leaves. The curves for the titrations of juice from the frozen variegated leaves are strikingly similar to those for the juice from fresh leaves of the green variety.

In the case of the titrations of fresh leaves of "*medio-picta,*" of which representative curves are shown in text-fig. 6, there is some indication that buffer action is least in chlorotic areas and greatest in entire leaves; the curves for green areas and entire leaves, however, were similar. This difference is not evident in titrations of fresh leaves of "*aurea.*" This may be due to the fact that only three titrations were made of fresh material in the latter case.

Smith ('26) has observed that the invisible rays of ultra-violet light from a quartz mercury vapor lamp caused a masking of the chlorotic symptoms on tobacco plants infected with true mosaic disease. It would be of interest to subject *Abutilon Thompsonii* to ultra-violet radiations through a series of glass filters the transmissions of which are known.

There have been no experiments recorded, in the literature or in the present study, to determine whether or not mottling on the leaves of *Abutilon* bears any direct relation to temperature. This subject has received the attention of physiologists dealing with tobacco and potato mosaics, and hence would be of interest in connection with these studies.

Baur's attempts to grow variegated plants of *Abutilon* in an atmosphere of air in which the carbon dioxide had been removed should be repeated in such a way as to permit a carbon dioxide-free air to be circulated and constantly renewed.

SUMMARY

1. The observations of Baur ('06a) with reference to the effects which the quality of light had upon certain plants infected with chlorosis could not be confirmed in the case of *Evonymus japonica* var. "*aurea.*" While the leaves of plants of *Abutilon Thompsonii* were observed to become quite uniformly green during the five-months exposure to sunlight deficient in blue rays under one set of conditions and green rays under another, the author is inclined

to favor the view that these effects may be attributed partially or entirely to shade which was produced under Corning's glasses Noviol "C" and G 34.

2. Continuous illumination for two months under experimentally controlled conditions where the intensity of light closely approached that of sunlight throughout the duration of the experiments, did not materially or permanently alter the typical variegated appearances of *Abutilon Thompsonii*, *Evonymus japonica* vars. "*aurea*" and "*medio-picta*" as compared with similar plants held under usual greenhouse conditions for the same interval of time. *Abutilon Thompsonii* plants receiving short exposures to artificial light under controlled conditions such as five-hour day and seven-hour day for an equal period of two months were observed to develop new leaves which became successively more uniformly green. Near the end of the experiments new leaves were maturing entirely without a chlorotic condition. It is possible that this should be said with some reservation, as here and there very minute chlorophyll-free specks, just visible to the unaided eye, could be observed on some leaves, which specks did not appear on control plants with uniformly green leaves when they were first placed under the conditions of the short-day experiments. Photographs showing these different effects are included in pl. 9.

3. Exposures of plants of *A. Thompsonii* to total darkness for intervals of time varying from several days to two weeks, during which time the stems became entirely defoliated of mature leaves resulted in the total loss of variegation in new leaves which were formed while the plants were in the dark, but matured in the light. On the other hand, leaves which developed after the plants were restored to the light became infected.

4. Studies of *Abutilon Thompsonii* made upon fixed and stained sections of areas transitional between green and chlorotic regions of strongly variegated leaves show little contrasting differentiation. No X-bodies were found in chlorotic, green, or transitional areas of the variegated leaves. Light treatments resulted in striking morphological modifications in leaf structure.

5. All attempts have failed to transmit the infectious chloroses by any other means than by grafting.

6. Successful transmission was obtained by grafting and budding *Evonymus japonica* var. "*aurea*" with green *Evonymus japonica*.

7. By stripping off successive crops of variegated leaves, *Abutilon Thompsonii* plants were made to develop uniformly green leaves, thus substantiating the work of Baur ('06a).

8. Electrometric determinations of hydrogen-ion concentrations were made by the quinhydrone electrode method, modified to accommodate determinations made in single drops of expressed juice, on crushed leaves of *Evonymus japonica* vars. "*aurea*," "*argenteo*," and "*medio-picta*," as well as leaves of a green variety. The method was found accurate within .03 pH. The following results were obtained: Chlorotic areas of all variegated varieties except "*aurea*" were higher in initial acidity than were the green areas. It has been observed that the decrease in pH of juice expressed from both chlorotic and green areas is in general directly proportional to the time in minutes of exposure of the juice to air. Such changes appear to be of sufficient magnitude to warrant their consideration in attempts to transmit the chlorosis of *Evonymus* by other means than grafting.

9. Electrometric determinations of total acidity were made on juice from these tissues by an apparatus which would permit the accurate titration of 2-cc. quantities. It was found that freezing the leaves before expressing the juice caused increase in buffer action of juices from green and variegated varieties of *Evonymus*. Freezing the tissue should be guarded against in inoculation experiments. There was a pronounced increase in total acidity of samples taken from the leaves of the green variety and from the green areas of the variegated leaves but not of samples taken from the chlorotic areas, when the expressed juice was exposed to air for four to twelve hours. It is believed that this difference between the behavior of the green and chlorotic areas has a theoretical significance.

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Explanation of Plate

PLATE 5

Semi-diagrammatic camera-lucida drawings of cross-sections of leaves of *A. Thompsonii*, showing the effect of light treatment.

Fig. 1. Condition after fourteen days of the experiment. Notice increased size of cells, spaces, thickness of leaf section, and abundance of chloroplasts. Continuous illumination of sunlight and gantry crane.

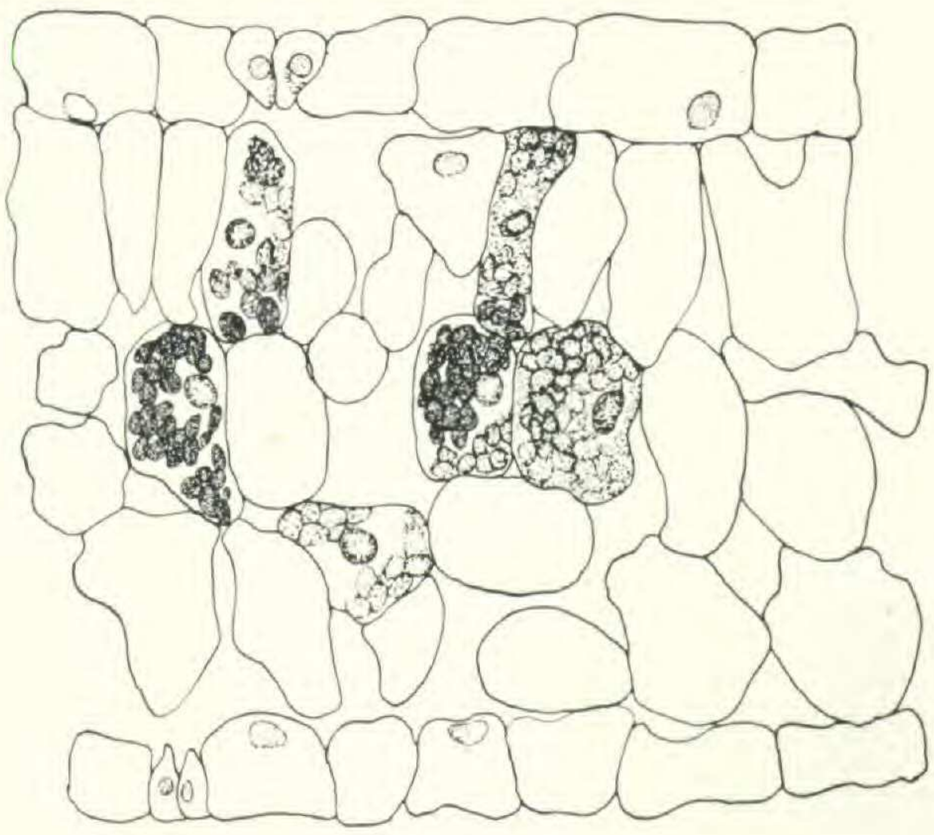
Fig. 2. Condition at beginning of experiment. Notice presence of unusually large intercellular spaces. Continuous illumination of sunlight and gantry crane.

Fig. 3. Chlorotic area at beginning of experiment. Continuous artificial illumination.

Fig. 4. Green area (?) of leaf drawn from same section as used in fig. 3.

Fig. 5. Condition at beginning of experiment, five-hour day.

Figs. 6 and 7. Condition fourteen days later, five-hour day. Notice modifications as opposed to those under continuous illumination.



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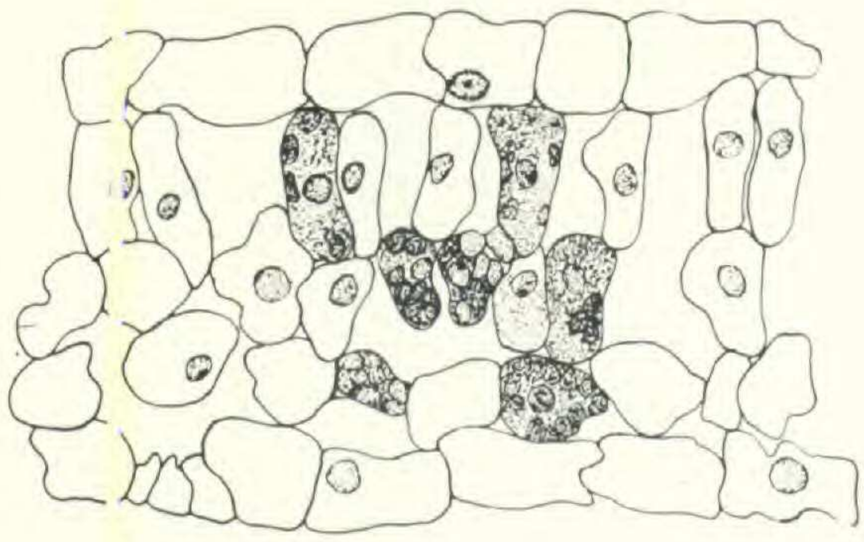

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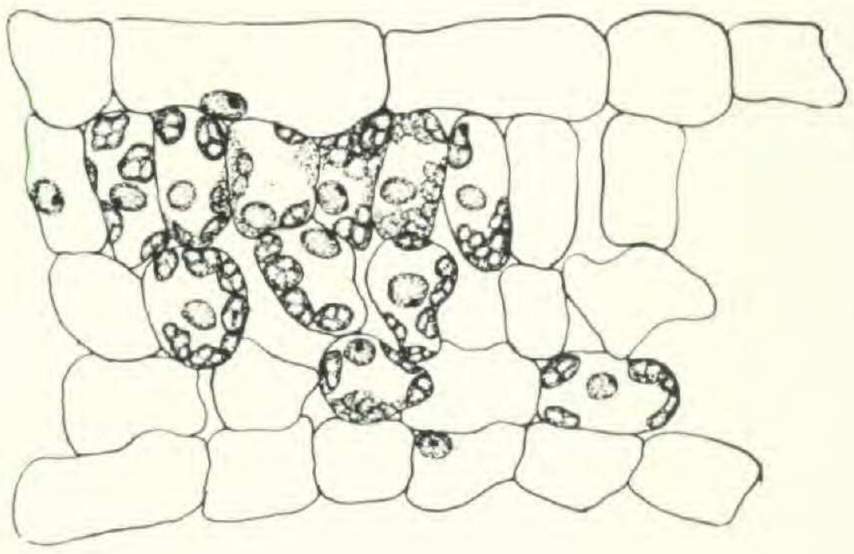

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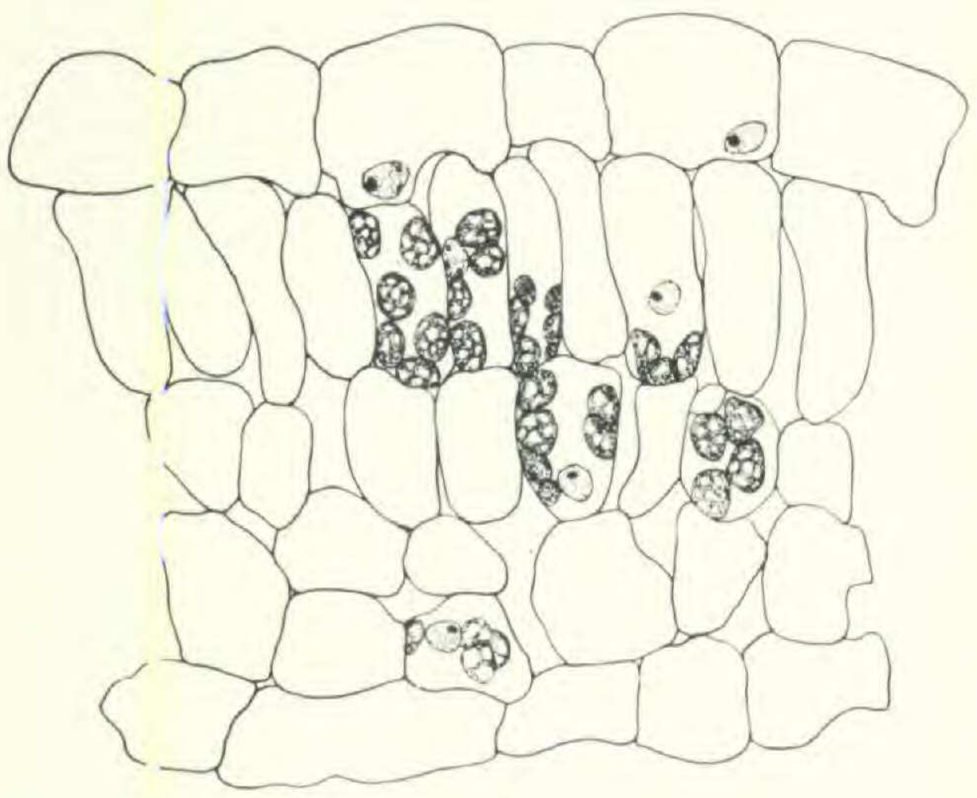

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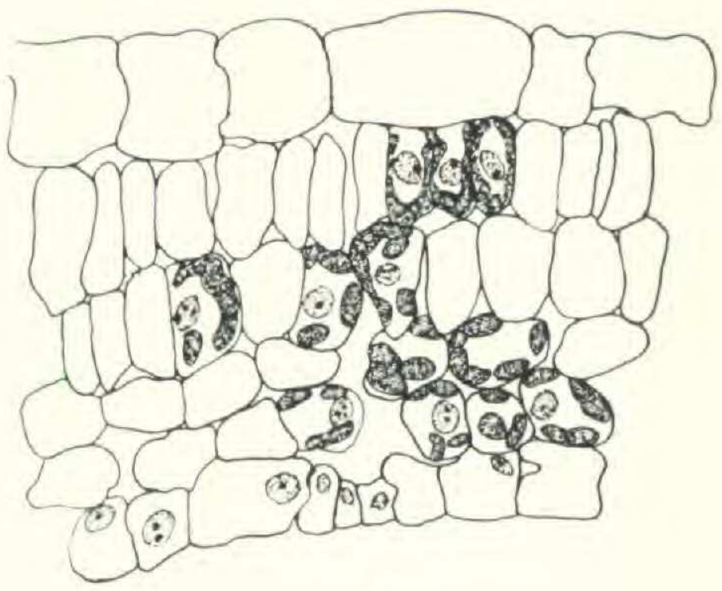

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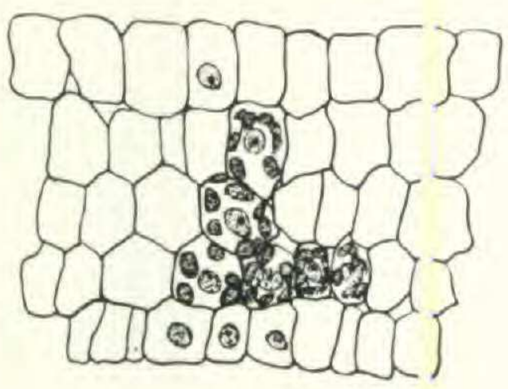

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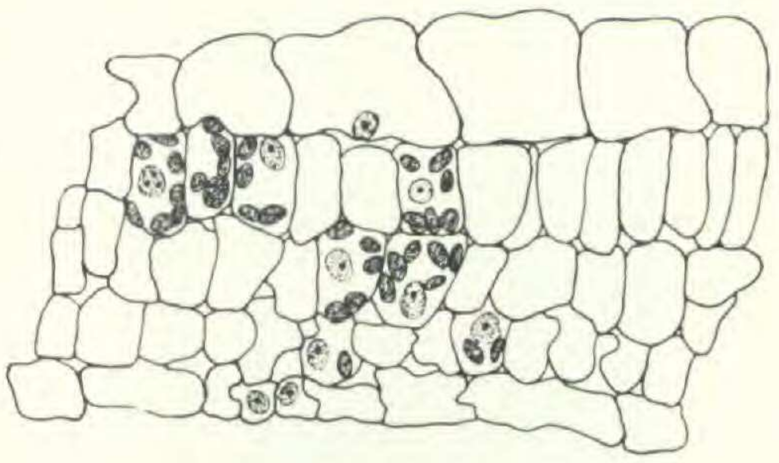

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Explanation of Plate

PLATE 6

Fig. 1. Plants of *Abutilon striatum* var. *Thompsonii*, variegated at beginning of experiment on light.

Fig. 2. Variegated leaves of *Abutilon Thompsonii*, showing typical mosaic pattern resulting from infectious chlorosis. Notice that the larger veins as well as some of the smaller ones limit the extent of the chlorotic areas.

Fig. 3. Variegated plants of *Evonymus japonica* at beginning of experiment on light.

1

2

3

DAVIS—INFECTIOUS CHLOROSIS

EXPLANATION OF PLATE

PLATE 7

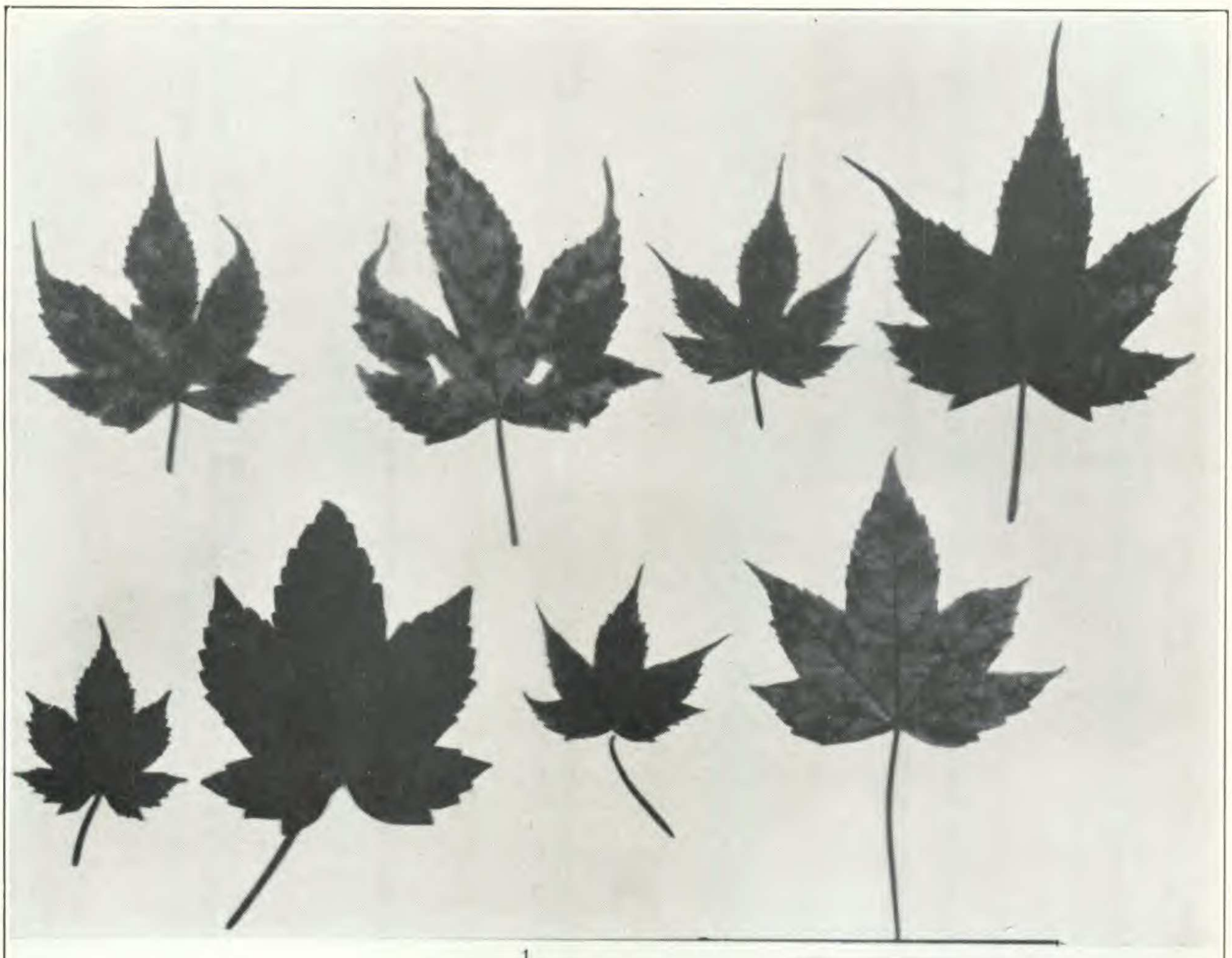
Fig. 1. New leaves of *A. Thompsonii*, showing effect of continuous artificial illumination for ten days, compared with leaves from control plants.

Top, left to right: 1, 1-ranked leaf from control plant; 2, 2-ranked leaf from control plant; 3, 1-ranked leaf under continuous artificial illumination after ten days; 4, 2-ranked leaf under continuous artificial illumination after ten days.

Bottom, left to right: 1, 1-ranked normal green leaf; 2, 2-ranked normal green leaf; 3, 1-ranked leaf under continuous artificial illumination after ten days; 4, 2-ranked leaf under continuous illumination after ten days.

Fig. 2. Showing transmission of infectious chlorosis by grafting experiments: 1, *Abutilon Thompsonii*, variegated; 2, *Kitaibelia vitifolia*, green; 3, *Kitaibelia vitifolia*, variegated, showing infection through grafting. (After Lindemuth ('99a), fig. 70).

Fig. 3. Showing transmission of infectious chlorosis by grafting experiments: *Althaea rosea* root graft in union with the stem of *Abutilon Thompsonii*, from which it became infested with chlorosis. (After Lindemuth ('07), fig. 50).



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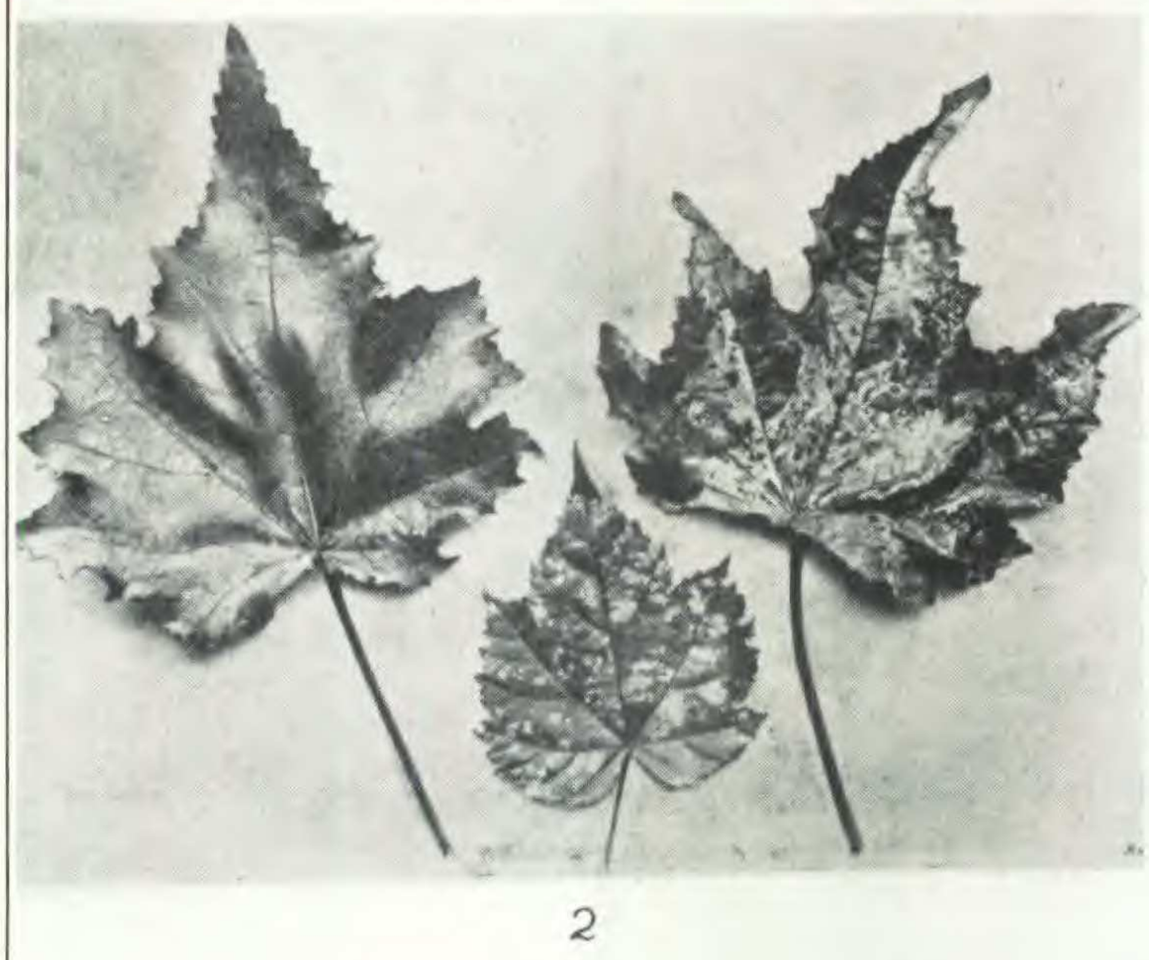

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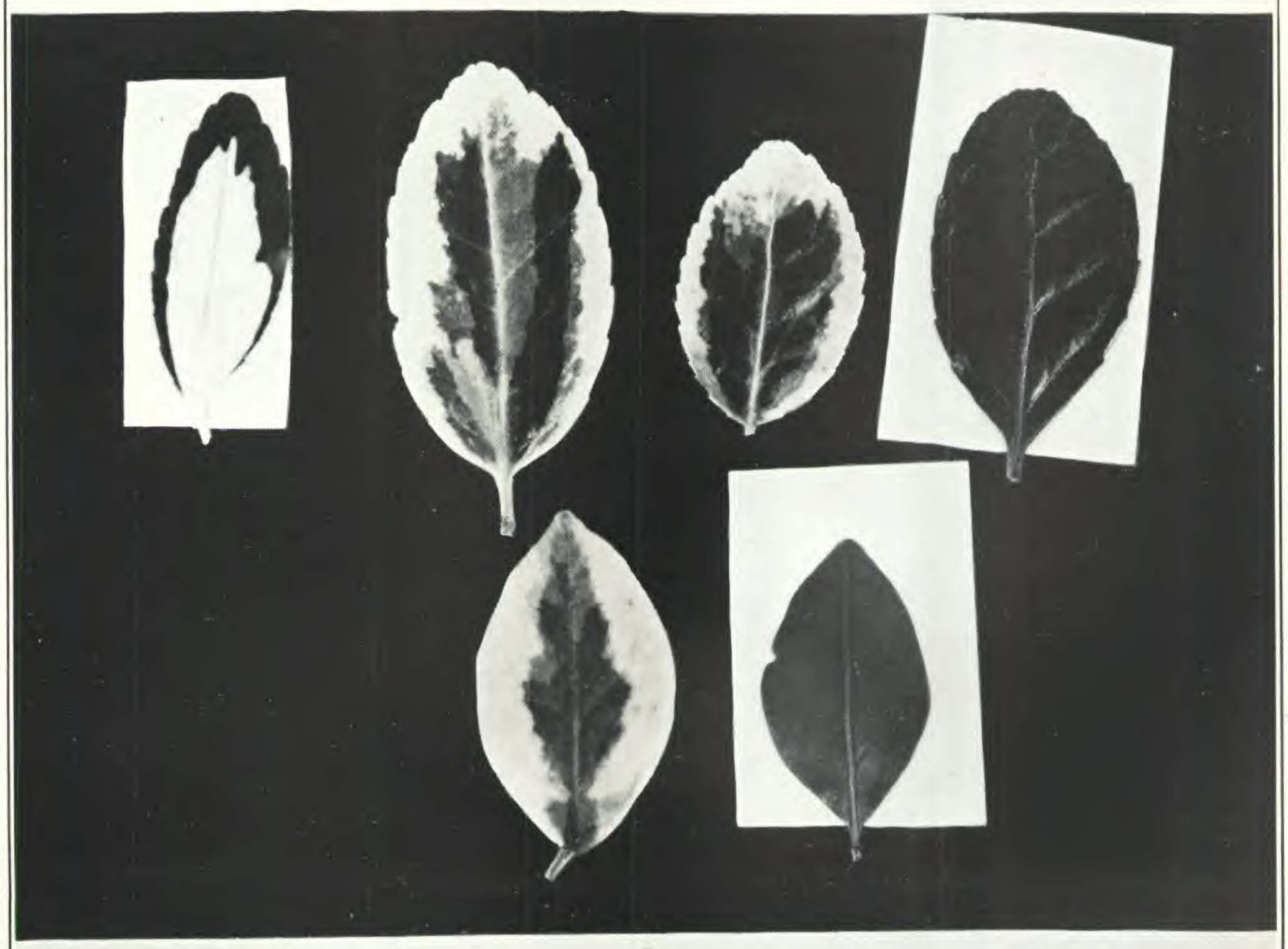
Explanation of Plate

PLATE 8

Fig. 1. *Abutilon megapotamicum* showing infectious chlorosis.
Fig. 2. Variegated leaves of *Evonymus japonica* varieties.
Top, left to right: 1, *E. japonica* var. "*medio-picta*"; 2 and 3, *E. japonica* var. "*argenteo*"; 4, *E. japonica* var. green.
Bottom, left to right: 1, *E. japonica* var. "*aurea*"; 2, *E. japonica* var. green.

1



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Explanation of Plate

PLATE 9

Fig. 1. *Abutilon Thompsonii*, showing effects of five-hour-day treatment.
Left to right: 173, *Abutilon* var. green, not infected; 28, 19, 8, 25, *Abutilon Thompsonii* variegated, showing loss of variegation among top leaves which developed during two months five-hour-day treatment; 120, 12, *Abutilon Thompsonii*, variegated, as they were under ordinary greenhouse conditions.

Fig. 2. *Abutilon Thompsonii*, showing effects of seven-hour-day treatment.
Left to right: 169, *Abutilon* var. green, from control greenhouse, not infected; 124, 94, 45, 120, *Abutilon Thompsonii* variegated, showing some loss of variegation among top leaves which developed during two months seven-hour-day treatment; 12, *Abutilon Thompsonii* variegated as in the control greenhouse; 173, *Abutilon* var. green, from control greenhouse.

1

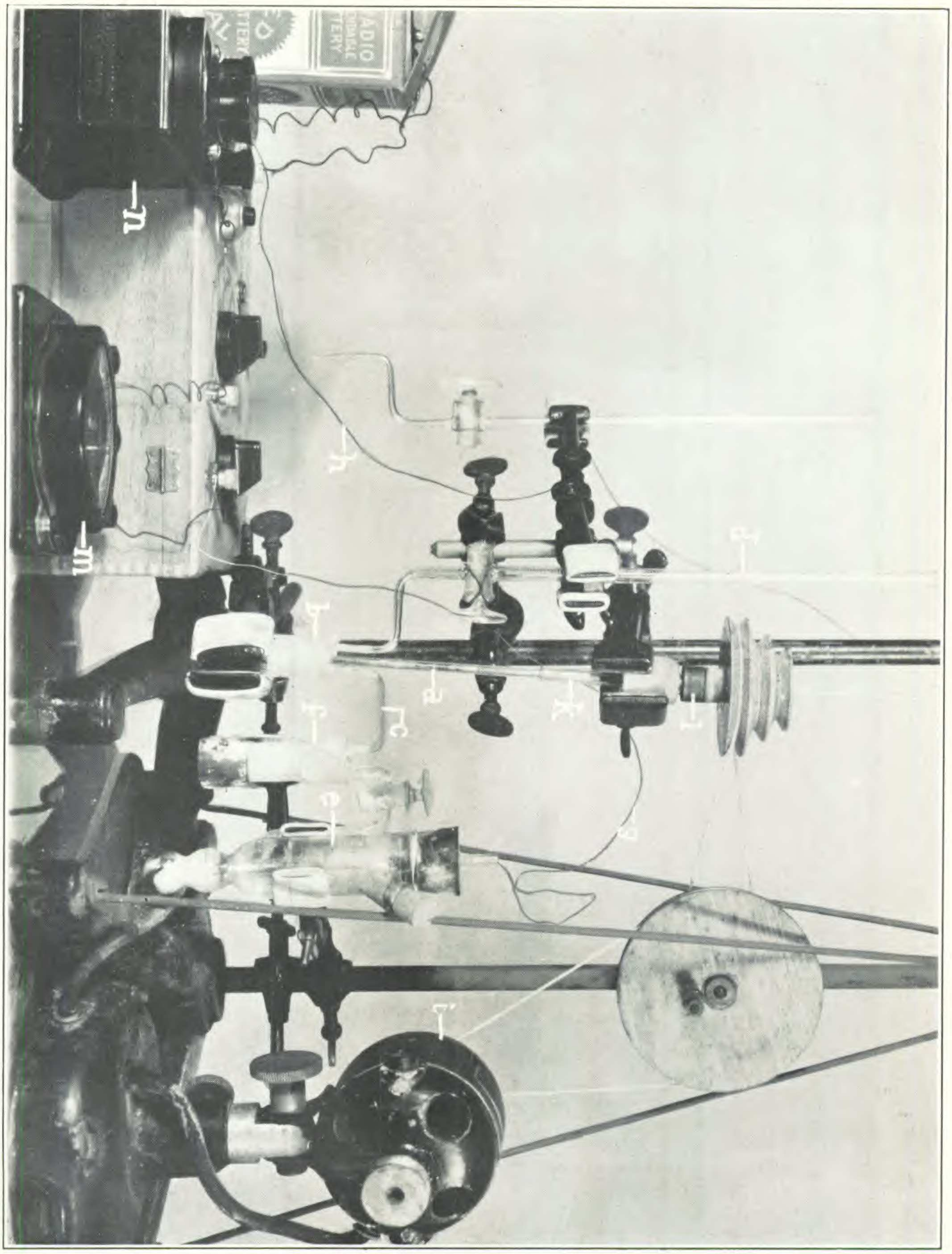
2

DAVIS—INFECTIOUS CHLOROSIS

Explanation of Plate

PLATE 10

Electrometric titration outfit which was used in the acidity experiments: *a*, glass tubing used for making connection with the platinum electrode, used also as a shaft for the stirring device; *b*, vial holding the sample to be titrated against standard alkali; *c*, salt bridge containing saturated KCl solution; *d*, burette, 2 cc. capacity; *e*, vessel containing a saturated calomel reference electrode; *f*, vial containing saturated KCl solution; *g*, wire leading from voltmeter to calomel electrode; *h*, wire leading from galvometer to quinhydrone-platinum electrode system; *i*, electric motor; *k*, sleeve of glass tubing for bearing; *l*, metal washer; *m*, millivoltmeter; *n*, galvanometer.



DAVIS—INFECTIOUS CHLOROSIS

Explanation of Plate

PLATE 11

Fig. 1. *Abutilon Thompsonii*, showing effect of removing successive crops of variegated leaves: 1, variegated leaves permitted to remain attached; 2, all variegated leaves removed as they developed. Notice that the smooth, deep green leaves of this plant are all free from chlorosis.

Fig. 2. Detached leaves of *Evonymus japonica* vars. "*aurea*," "*medio-picta*," and a green variety showing infection of the green: 1, Showing infection with chlorosis resulting from graft with "*aurea*." Notice very inconspicuous mottling of the leaves; 2, Variety *medio-picta*, a type of non-infectious variegation; 3, Showing leaf of "*aurea*" variety which transmitted a chlorosis to the green; 4, Showing infection resulting from a bud of "*aurea*" which was inserted into the bark of the green individual.